Study to Evaluate the Effects of BKR-017 on Insulin Sensitivity and Triglycerides in Type 1 Diabetes Subjects

NCT03999853

13July2023

CL-301

Study to Evaluate the Effects of BKR-017 on Insulin Sensitivity and Triglycerides in Type 1 Diabetes Subjects

PROTOCOL DATE:

July 13, 2023Amendment 5

Protocol Version 6

Revision History		
Protocol	**Date**	**Version Number**
Original Protocol	March 1, 2020	1.0
Amendment 1	May 20, 2022	2.0
Amendment 2	July 18, 2022	3.0
Amendment 3	December 5, 2022	4.0
Amendment 4	April 25, 2023	5.0

Section	Change Summary
Title Page	Protocol Date changed to July 13, 2023
TOC & Page Numbers	Appropriate page number changes
Footer and synopsis page	Changed Ver. 5 to 6where appropriate
Signature Page	Protocol Date changed to July 13, 2023D, added version
Baseline Visit: Screen (Day -28)	In addition, study CGM sensors will be dispensed with subject instructions on use of Dexcom CLARITY app to capture glucose data throughout study participation. Diary cards will be mailed/emailed to the study team every 7 to10 days throughout the study. Subjects using an insulin pump, will be advised to mail/email a copy of their pump settings and insulin doses every 7 to10 days or upload their pump data to a prespecified, linked online account compatible with their pump. Height will be measured at screen visit. Urine pregnancy test added. An eight-week supply of investigational product (BKR-017) will be dispensed. Subjects will not start dosing until Day 0. Females of childbearing potential and able to become pregnant, must agree to use one of the birth control methods listed in the consent form, for the duration of the study.
Treatment Period: Day 0 to 48	Study Diary cards will be mailed/emailed to the study team every 7 to10 days throughout the study. .
Visit 2: Final Visit (Day 48)	Height will be measured.

TABLE OF CONTENTS

LIST OF TABLES

ABBREVIATIONS

AE	Adverse Event
ADG	Average Daily Glucose
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
API	Active Pharmaceutical Ingredient
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CGM	Continuous Glucose Monitoring
CPR	C-Peptide
eCRF	Electronic Case Report Form
eGDR	Estimated Glucose Disposal Rate
GLP-1	Glucagon-Like Peptide-1
HbA1c	Hemoglobin A1c
HDPE	High Density Polyethylene
HIPAA	Health Insurance Portability and Accountability Act
HOMA-IR	Homeostatic Model Assessment and Insulin Resistance
hs-CRP	High-sensitivity C-Reactive Protein Hip-to-Waist Ratio
HWR	Hip-to-Waist Ratio
HTN	Hypertension
ICF	Informed Consent Form
ITT	Intent-To-Treat
IRB	Institutional Review Board
OPC	Out-Patient Clinic
PI	Principle Investigator
SAP	Statistical Analysis Plan
T1D	Type 1 Diabetes
USP	United States Pharmacopeia
1,5-AG	1,5-Anhydroglucitol

SIGNATURE PAGE

Study Title:	Study to Evaluate the Effects of BKR-017 on Insulin Sensitivity and Triglycerides in Type 1 Diabetes Subjects
Protocol Date and Version:	July 13, 2023Amendment5, Version6
Protocol Number:	CL-301
Investigator:	Adrian Vella, MD Mayo Clinic 200 1st St SW Rochester, MN 55905
Institutional Review Board:	Mayo Clinic Institutional Review Board [redacted] 200 First Street SW Rochester, Minnesota 55905

We, the undersigned, have carefully read this protocol and agree that it contains all the necessary information required to conduct the trial and that it complies with principles of Declaration of Helsinki.

Investigator:

Adrian Vella, MD

_____ _____

Signature Date (month/dd/year)

CLINICAL PROTOCOL SYNOPSIS	Protocol No.:	CL-301
Sponsor: Mayo Clinic	**Investigator:** Adrian Vella, MD	

Title of Study: Study to Evaluate the Effects of BKR-017 on Insulin Sensitivity and Triglycerides in Type 1 Diabetes Subjects

Study Center: Mayo Clinic, Rochester, MN	**Country:** US

Objectives: To evaluate effect of colon-targeted butyrate tablets (BKR-017) on insulin sensitivity and triglycerides in type 1 diabetes subjects.

					Number of Tablets		Dose	
Test Group	**Test Product**	**No. of Subjects**	**Dose Regimen**	**Dosing Duration (days)**	**Each dose (tablets)**	**Daily (tablets)**	**Single Dose**	**Total Daily Dose**
1	Active	16	BID	48	3	6	1.5 g	3.0 g

Test Product: BKR-017 is an oral tablet formulation designed to target delivery of butyrate to the colon via a colon-targeting technology. [redacted] The tablets will be supplied by BioKier in 150-ml high density polyethylene (HDPE) white bottles.

Design: This study will test effect of BKR-017 (colon-targeted 500 mg butyrate tablets) as adjuvant therapy in type 1 diabetes (TID) Subjects on metabolic control in this population. Insulin sensitivity, insulin usage, glucose control (variability), and triglycerides will be measured after 7 weeks of treatment and compared to Day -28.

Number of Subjects: Assuming a 30% (approximate) early discontinuation rate, 16 Subjects will be consented and enrolled to ensure that 13 Subjects complete the 11-week study (4-week run in and 7 -week treatment period).

Diagnosis and Main Criteria for Inclusion: Criteria for Subject recruitment will be: 20–80 years of age; type 1 diabetes with C-Peptide (CPR) less than 0.5 ng/mL; HbA1c level of 6.4–8.9%; at week -4. The study design will be a single-arm, open-label study. Subjects will be expected to use the Dexcom G6 Continuous Glucose Monitor (CGM) system and be willing to share their data via the CLARITY app with the study team.

Duration of Subject Enrollment: Eleven (11) weeks; baseline period 28 days (4 weeks), treatment period: 48 days (7 weeks). Two visits will be required: one screen visit and one visit after 48 days of treatment.

STUDY PERIOD (See Time and Events Schedule, Section 6)

Baseline Period (Day -28):

Visit 1 (Day -28): Following web- and phone-based pre-screening, subjects who remain eligible will be scheduled for a screening visit. Subjects will be asked to arrive for the screening visit (Visit 1) in a fasted state (10-hour fast). Subjects will be consented prior to obtaining any biological samples or performing any study procedures. Subject eligibility will be assessed, demographics, medical history, and concomitant medications will be obtained along with- height, weight, waist and hip circumference to calculate Hip-to-Waist Ratio (HWR), and an abbreviated physical exam will be performed (including signs for edema). Fasting samples will be obtained for glucose and triglycerides. Additional blood samples will be obtained for HbA1c, routine hematology, and routine chemistry assessments. Hypertension (HTN) will be determined from medical history and concomitant medications. Blood pressure will be measured and used, along with HbA1c, to calculate the eGDR (estimated Glucose Disposal Rate). A urine pregnancy test will be obtained for females of childbearing potential.

After signing consent and meeting all inclusion criteria, subjects will be instructed on, and supplied with a study provided Dexcom G6 sensors , to collect glucose data. Subjects will be instructed to keep their Dexcom CLARITY app active, so that glucose monitoring data can be accessed by the study team throughout the study. Diary cards will be provided for the subject to record insulin doses, changes to concomitant medications and adverse events. Diary cards will be mailed/emailed to the study team every 7 to10 days throughout the study. Subjects using an insulin pump, will be advised to mail/email a copy of their pump settings and insulin doses every 7 to10 days or upload their pump data to a prespecified, linked online account compatible with their pump (i.e. Medtronic CareLink, Tandem T Connect or Glooko), whereby the relevant data can be abstracted by the study team.

An eight-week supply of investigational product (BKR-017) will be provided to subjects. Subjects will not start dosing until Day 0.

During the entire study, subjects will be asked to maintain their daily routines, including diet and exercise. Females of childbearing potential and able to become pregnant, must agree to use one of the birth control methods listed in the consent form, for the duration of the study.

Treatment Period: Day 0 to Day 48:

Day 0 will occur approximately 28 days after the screen visit (agreed upon with the subject). The study team will communicate with the subject via a phone call or virtual visit on this day. After ensuring that the CGM data collected is acceptable, subjects will be instructed to commence taking the investigational product (BKR-017) as instructed.

Subjects will be instructed to keep their Dexcom CLARITY app active, so that glucose monitoring data can be accessed by the study team. Subjects will continue to fill out diary cards, to record insulin doses, changes to concomitant medications and adverse events. Diary cards will be mailed/emailed to the study team every 7 to 10 days. Subjects using an insulin pump, will be advised to mail/email a copy of their pump settings and insulin doses every 7 to 10 days or upload their pump data to a prespecified, linked online account compatible with their pump (i.e., Medtronic CareLink, Tandem T Connect or Glooko), whereby the relevant data can be abstracted by the study team.

Subjects will be contacted by the study team on Days 18 and 28, to assess study compliance, review concomitant meds and adverse events.

Final Visit (Day 48):

At the final study visit, **Visit 2** (**Day 48)**, fasting samples (10-hour fast) will be obtained for glucose and triglycerides. Additional blood samples will be obtained for HbA1c, routine hematology, routine chemistry assessments, CPR, and the exploratory tests 1,5 AG and hs-CRP. Blood pressure, height, weight, waist and hip circumference to calculate HWR, be obtained and an abbreviated physical exam will be performed. A urine

pregnancy test will be obtained for females of childbearing potential. The CGM sensor will be removed, and data downloaded.

Subjects will be required to return the study CGM sensor, all unused test product in the original container along with any diary cards at this visit. The diary card and returned product will be reconciled by the study staff, and medication changes and adverse events recorded on the diary will be reviewed.

Subjects with the following conditions will be excluded: History of bariatric or intestinal surgery, currently pregnant, nursing, or trying to become pregnant, type 2 diabetes, active gastrointestinal disease (e.g., irritable bowel syndrome, inflammatory bowel disease (e.g., ulcerative colitis, Crohn's Disease, diverticulitis, gastroparesis, chronic/frequent diarrhea or chronic/frequent constipation), active and clinically significant hepatic, renal, or pancreatic disease, history of congestive heart failure, prior MI or significant cardiovascular disease, hepatic, renal, or pancreatic disease, history of congestive heart failure, prior MI or significant cardiovascular disease, uncontrolled hypertension uncontrolled hyper- or hypothyroid disease, active infection or history of trolled hypertension uncontrolled hyper- or hypothyroid disease, active infection or history of hepatic, renal, or pancreatic disease, history of congestive heart failure, prior MI or significant cardiovascular disease, uncontrolled hypertension uncontrolled hyper- or hypothyroid disease, active infection or history of chronic infection, subjects reporting an anticipated change in exercise or diet, subjects on a high fiber diet, subjects with pitting edema, and subjects who, in the investigator's judgment, are not suitable for the study for any other reason, or subjects who cannot commit to the requirements of the study.

Subjects currently taking the following therapies are excluded: GLP-1 receptor agonists (e.g., Byetta®, Bydureon®, Adlyxin®, Victoza®, Saxenda®, Trulicity®, Tanzeum®, Ozempic®), Amylin analogs (e.g., Symlin®), Thiazolidinediones (e.g., Actos®, Avandia®) and any combination of these medications (e.g., Avandamet®, Avandaryl®, Duetact®, Actoplus Met®, PioMet®), Acarbose (Glucobay®), Miglitol (Glyset®). Additionally, subjects taking oral and injectable corticosteroids (e.g., prednisone), oral and injectable antibiotics, opioids and probiotics or other medications that alter gastrointestinal motility, chemotherapy, radiation, cytotoxic therapy, immune suppressants, antiepileptic drugs, colesevelam (Welchol®), and other supplements containing butyrate are also excluded from the study.

Efficacy and Safety Evaluations	
Primary	Insulin sensitivity, as measured by the estimated glucose disposal rate (eGDR), a validated clinical tool for estimating insulin sensitivity in T1D
Secondary	The following secondary evaluations will be performed: 1. Glucose variability 2. Triglycerides 3. Insulin usage, as reported on diary card
Safety	Blood pressure, physical exam, chemistry, hematology and treatment-emergent adverse events
Exploratory	1,5 AG and hs-CRP

CLINICAL PROTOCOL SYNOPSIS		Protocol No.:	CL-301
Sponsor: Mayo Clinic		Investigator: Adrian Vella, MD	
Statistical Analysis of Primary Endpoint:	**Primary and Secondary Efficacy Data**: The primary endpoint is the change from baseline (Day 0) to Day 48 in insulin sensitivity as measured by eGDR. The secondary endpoint of glucose variability (as measured from CGM data) is also the change from baseline (Day 0) to Day 24 and Day 48. However, the secondary endpoint of lowering of triglycerides measurements will be calculated from the baseline at Day -28. **Safety Data**: Assessment of safety will be made using the change from baseline in vital signs, physical exam, chemistry, hematology, and treatment-emergent adverse events. The safety analysis set will include all Subjects that received any amount of study treatment. The safety analysis set will include all Subjects that received any amount of study treatment. **Sample Size Estimates**: Sample size estimates are based on practical considerations.		
Synopsis Date: July 18, 2022, Amendment 2, Version 3.0			

1. INTRODUCTION

Summary: BioKier, Inc. is developing colon-targeted, oral formulations of natural gut hormone secretagogues as non-prescription medical foods or supplements for nutritional use for improvement of insulin sensitivity in type 1 (T1D) and type 2 (T2D) diabetes. The natural compounds under investigation by BioKier are butyrate and L-glutamine. An earlier clinical study (CL-201), conducted at Pennington Biomedical Research Center, demonstrated that treatment for 4 weeks with the L-glutamine formulation (BKR-013) improved insulin sensitivity in a significant number of T2D subjects. BioKier now plans to investigate the effects of an oral butyrate tablet formulation (BKR-017) on insulin sensitivity and other metabolic parameters in diabetes patients; this study will be in T1D subjects. The long-term anti-diabetes effects of treatments that increase the presence of butyrate in the colon are well documented, which suggests that a colonic formulation of butyrate is a viable option for chronic use in both T1D and T2D. Along with L-glutamine, butyrate is one of the most active gut hormone secretagogues among natural compounds. Butyrate, a short-chain fatty acid, is the active ingredient of BKR-017 to be tested in the current clinical protocol (CL-301). The findings from the pilot study CL-201 with the L-glutamine capsule BKR-013 showed that insulin resistance is a relevant efficacy endpoint and therefore has been identified as the primary endpoint in this study.

Gut hormones, including glucagon-like peptide-1 (GLP-1), secreted by L-cells in the lower gut in response to nutrient stimulation, are important in the body's regulation of energy balance [1]. They signal the arrival of food in the gut to regulatory centers controlling glucose metabolism, appetite, and lipid metabolism. As well as regulating glucose homeostasis and satiety, an important effect of GLP-1 secreted from the gut is inhibition of fat absorption, which results in lower levels of free fatty acids and triglycerides in the circulation. Impaired secretion of gut hormones by L-cells in response to meals has been reported in obesity [2;3], T2D [3], and T1D [4], although in these conditions the L-cells retain their ability to respond to secretagogues. Also, available evidence indicates that GLP-1 receptor agonists improve glycemic control when used in conjunction with insulin in patients with T1D [5], suggesting that augmented GLP-1 secretion can be beneficial in T1D. Butyrate is one of the most active natural L-cell agonists, as shown in *in vitro* [6-8] and has been shown to be an active secretagogue of GLP-1 in *in vivo* studies [8]. There is a preponderance of human data demonstrating that the beneficial effect on diabetes of delivery of non-digestible carbohydrates to the colon are due to the actions of butyrate which is a product of fermentation of carbohydrates by colonic bacteria. Furthermore, the improvement in metabolic control after fermentation of ingested carbohydrates in the colon of rats [9] and humans [10] has been shown to be due to increased GLP-1 secretion. However, as effective as these approaches can be, they have not achieved wide therapeutic acceptance because the fermentation process causes serious GI side effects including diarrhea, bloating, and flatulence, which were not tolerated in clinical trials [11]. Alternate methods of delivering butyrate to the colon to achieve the same effects, but avoiding fermentation, were patented by BioKier.

In order to affect the metabolic conditions that result from impaired gut hormone secretion, BioKier has formulated the nutrient butyrate into a colon-targeted tablet formulation (BKR-017) intended to stimulate secretion of GLP-1 from L-cells in the lower gut. Butyrate delivered to the colon in tablet form will not cause the side-effects seen with formation of butyrate by fermentation.

It is well recognized in the scientific and medical community that stimulation of GLP-1 secretion by activation of L-cells in the lower gut is an area of expected dynamic development [12-14]. BioKier is utilizing the GLP-1 secretagogue properties of butyrate to develop this safe, natural compound as a medical food. While a number of metabolic conditions result from GLP-1 dysregulation, BioKier is evaluating the effect of BKR-017 on insulin sensitivity in T1D patients. In this population, BKR-017 is not intended to treat T1D, and as such would not replace insulin therapy, but rather BKR-017 will provide a nutritional support to any currently prescribed T1D therapy, which may involve prescription medication, or diet and exercise, or both. Specifically, we expect that use of BKR-017 will afford better glucose control including reduced variability and reduced frequency of hypoglycemic episodes. This would be due to improved insulin sensitivity which is expected to lead to lower insulin use in those patients and would cause weight loss in patients with metabolic obesity. Another benefit of lowering insulin resistance would be to lower cardiovascular risk as there is plenty of evidence associating insulin resistance with increased CV risk [15]. Elevated triglycerides are one of the validated risk factors for CV disease in both type 1 and type 2 diabetes patients [16], and one of the beneficial effects of metabolic surgery and colonic butyrate produced by fermentation is significant lowering of triglycerides and therefore reduction in CV risk [17;18]. Pertinently, GLP-1 receptor agonists [19] and DPP-4 inhibitors [20] that elevate endogenous GLP-1 have been shown to decrease cardiovascular risk and inflammatory markers including triglycerides, free fatty acids, VLDL-C, apoB-48, and ApoB (reviewed in [21]).

Butyrate, formulated as BKR-017 to target the colon, is being developed by BioKier as a medical food. Regulations require that medical foods be consumed or administered enterally under the supervision of a physician and that they are intended for the specific dietary management of a condition for which distinctive nutritional requirements, based on recognized scientific principles, are established by medical evaluation such as appropriate laboratory and clinical science. An Investigational New Drug (IND) application is not required to conduct clinical investigations of a medical food, and a New Drug Application is not required for marketing.

Previous studies conducted by BioKier, in which butyrate and L-glutamine was administered directly via infusion to the colon of T2D subjects, showed that single 1g doses of either butyrate or L-glutamine improved biomarker responses to an oral glucose challenge. Specifically, colonic administration of butyrate and L-glutamine both improved secretion of the glucoregulatory gut hormone GLP-1 and the subsequent insulin response. As follow-on to these effects observed on biomarkers, clinical outcome data is required to support marketing claims for BKR-017, the oral

formulation of butyrate, as a medical food, which would satisfy the FDA requirement of clinical data based on recognized scientific principles necessary for labeling a medical food. Note, in this study in T1D, it is not anticipated that improved GLP-1 secretion will necessarily increase insulin secretion but will improve insulin sensitivity, thus reducing insulin dose and glucose variability.

BioKier's Approach: While several nutrients have gut hormone secretagogue activity, BioKier has selected the nutrients butyrate and L-glutamine to formulate as candidates for medical foods to advance through clinical development. Although both have potent acute GLP-1-secretagogue activity, butyrate has well documented chronic effects on glucose regulation, including the reports of non-digestible carbohydrates improving metabolic control over the long term, discussed above. There is large body of scientific and clinical evidence supporting the role of butyrate in improving metabolic control in diabetes:

1. α-Glucosidase inhibitors, such as acarbose and miglitol, help control patients' glucose by shuttling saccharides to the colon where they are processed to butyrate and increase GLP-1 secretion [22;23]. That the increase in magnitude of improvement is in proportion to the increase in adverse gastrointestinal effects provides additional evidence that fermentation of saccharides to butyrate is the mechanism by which those drugs work and also why those drugs are not used, because of the adverse side-effects. An increase in colonic butyrate and augmented GLP-1 secretion were demonstrated in patients treated with those drugs.
2. Tagatose, which was in clinical trials [11], produces butyrate in the colon by fermentation. Major outcomes after 1 year of tagatose dosing in this study were:
    a. Mean HbA1c fell from 10.6% to 9.6%
    b. Body weight declined from 108.4 to 103.3 kg
    c. HDL cholesterol progressively rose from 30.5 to 41.7 mg/dL

    However, Tagatose, which was in development as anti-diabetes drug by Spherix, was not commercialized due to side-effects of fermentation.
3. Oligofructose supplementation, which produces butyrate in the colon by fermentation, resulted in weight loss and better glucose control [24]. Also, insulin decreased in the oligofructose group and increased in the control group between initial and final tests ($P < 0.05$), indicating that insulin sensitivity improved with treatment.

BioKier has developed the colon-targeted tablet formulation, BKR-017, to deliver butyrate to the site of action in the colon. The dose of butyrate delivered is at least as large as the amounts of butyrate that are very effective for diabetes patients when produced from products that deliver saccharides to the colon. BKR-017 is designed to protect butyrate from digestion and absorption in the stomach and upper digestive system and deliver it to the colon where it can activate the secretion of gut hormones from L-cells. A safety advantage of targeting the luminal surface of the gut epithelium is that use of a compound such as butyrate, with local activity and very short systemic half-life, will result in negligible change to systemic levels and negligible off-target

effects. Accordingly, there should not be interference or interactions with other medications that are released in the small intestine or that circulate in plasma.

[redacted]

BioKier's Previous Studies - Pre-Clinical: BioKier has completed acute and chronic exploratory pre-clinical studies. The studies provided proof of the concept that butyrate was able to restore oral glucose-induced GLP-1 secretion when administered to the colon of diabetic rats and, when dosed chronically in an oral, colon-targeted, sustained-release formulation, prevented the development of diabetes in a rat diabetes model. Effects noted were a reduction in basal glucose levels and an improvement in insulin sensitivity [8].

BioKier's Previous Studies – Clinical: BioKier has conducted acute studies in T2D patients in which single doses of either butyrate or L-glutamine were administered to the colon of subjects in an enema solution. In diabetes drug-naïve patients, both butyrate and L-glutamine were effective in restoring oral glucose-induced secretion of GLP-1 and insulin to levels approaching those seen in non-diabetic controls [8].

BioKier has also conducted a 4-week clinical study (CL-201 at Pennington BioMedical Research Center) in T2D subjects to determine effect of an oral formulation of L-glutamine on glucose levels and insulin sensitivity. The exploratory clinical investigation employed a two-way crossover design, in which a BID dose of 1.5g L-glutamine in colon-targeted capsules (BRK-013), or placebo, were administered orally to T2D subjects. After 4 weeks of treatment with colon-targeted L-glutamine, which has the same mechanism of action as butyrate, there was an improvement in insulin sensitivity and lowering both fasting glucose and insulin in a significant number of patients. Significantly, in six of nine subjects there was a marked improvement in insulin sensitivity -- HOMA IR changed from 2.86 to 1.67 (n=6) in the responders, which compares favorably to 2.75 to 1.27 (n=10) reduction for bariatric surgery patients [26]. These data supported the concept that chronic delivery of a natural gut hormone secretagogue to the colon of diabetes patients has

potential as a therapeutic in T1D, as well as T2D, because metabolic surgery and colonic fermentation of carbohydrates is beneficial in both T1D and T2D.

Safety: Butyrate is a simple chemical entity, stable and readily available in United States Pharmacopeia (USP) grade and Active Pharmaceutical Ingredient grade (i.e., suitable for human use).

BioKier has conducted IRB-approved clinical studies of butyrate in T2D patients; notably, three single-dose infusion studies under Western IRB approval. Furthermore, a study done at the Mayo Clinic in 1993 investigated the effects of butyric acid and glutamine delivered directly to the colon of pouchitis patients by suppository [27]. Butyric acid (4.4g bid and glutamine 1g bid) were given to patients for three weeks. The study reported positive effects on pouchitis but, significantly, found no safety issues of doses higher than those proposed by BioKier. In the combined clinical studies conducted by BioKier, there were 30 subjects exposed to 1g butyrate. Additionally, in a BioKier-sponsored study at the Mayo Clinic in which Dr. Vella was the Principal Investigator, 4 patients were treated with butyrate suppositories at 3g a day for 4 weeks with no adverse effects observed. Butyrate is also used as an enema in doses up to 8g for treatment of colon inflammatory conditions. Furthermore, the safety of butyrate supplementation of enteral or parenteral nutrition has been widely studied in chronic conditions (solid tumors) at doses of 14-28g/d. For Crohn's disease it was determined that 4 g/day butyrate as enteric-coated tablets for 8 weeks [28] was safe and well tolerated. Butyrate toxicity has been studied in humans and it was determined that dose-limiting toxicity was not identified [29]. The escalation was halted at 200 mg/kg (of butyrin, a butyrate prodrug) three times daily (40 g per day) as further increases were felt to be not practical due to the large number of capsules which would need to be administered. In another study [27] butyric acid (4.4g BID) was given to patients for three weeks without any adverse effects. See Appendix 1 for human use summary.

A safety advantage of BKR-017 over administration of large oral or parental doses of butyrate is that it will lead to minimal systemic exposure to butyrate because it is released slowly, acts locally in the colon, and is metabolized quickly.

Rationale for the current study design: Butyrate was selected as a candidate compound based on clinical data from studies with non-digestible saccharides (tagatose, lactitol, and oligofructose), and α-glucosidase inhibitors; both approaches result in fermentation of saccharides by butyrogenic bacteria in the colon, producing butyrate and augmenting GLP-1 secretion. Long-term treatment with these products has been shown to be beneficial for diabetes patients, both T1D and T2D.

A BID dosing regimen was selected because the tablet core is designed to release butyrate in the ascending colon over a period of eight or more hours. This timing of the sustained-release profile suggests that BID dosing will be necessary to achieve optimal effects.

The 1.5g dose of BKR-017 was selected based on several factors including: safety data from BioKier studies and other relevant studies; practical considerations (three 500mg tablets was determined to be the highest number of tablets that could practicably be administered as a single dose); BioKier's efficacy data from single intracolonic dosing in which 1.0g was effective in increasing GLP-1, in diabetes drug-naïve patients; and on the relative activities of nutrients and gastrointestinal compounds reported in published studies. Considerations included:

1. Fiber producing ~1g of butyrate in the colon was effective in improving glucose regulation [9;9] (BioKier anecdotal data).
2. Comparison of concentration of butyrate produced in the colon after use of maximal dose of acarbose and amount of butyrate release from BKR-017[30];
3. The amount of colon-infused bile acids effective in induction of GLP-1 secretion [31]; and
4. The amount of bile acids shuttled to the colon by Welchol [32].

The proposed dose for this study has been increased from the 1.0-gram dose used in the single-dose studies to 1.5g to account for the lower concentrations of butyrate that will result from the sustained release profile of the BKR-017 tablets. Even at 1.5g BID (3.0g total daily dose), this dose is within the practical range for repeated dosing and well within dose and duration ranges safely administered in clinical studies and currently used in clinical practice (see Appendix 1: Previous human experience with butyrate [selected studies]).

Considering that BKR-017 will require chronic dosing to supplement a T2D patient's prescribed diabetes treatment in order to maintain adequate insulin sensitivity, longer-term safety should be taken into consideration. A seven -week duration of dosing was selected based on the safety data from reported clinical investigations (see Appendix 1) and this is within the duration of dosing of the butyrate in reported clinical trials. Furthermore, this duration of dosing was selected in order to cross reference the "placebo effect" observed in T2D patients. This placebo effect, which is known to affect daily glucose levels, has been shown to diminish by approximately 3 weeks [33]. Therefore, in consideration of safety data and practical considerations a seven -week dosing regimen was selected.

2. STUDY PURPOSE AND OBJECTIVES

This study will test the hypothesis that delivery of the butyrate-containing BKR-017 tablet to the colon of T1D subjects will improve insulin sensitivity. Additionally, the effect of BKR-017 on triglycerides and glucose variability will be assessed. The safety and tolerability of repeat dosing (up to 48 days of administration) of BKR-017 will be determined. By exploration of the effects of BKR-017 on various metabolic biomarkers, the data generated from this study will be used to support continued evaluations of BKR-017 as non-prescription product for T1D patients requiring

nutritional support to improve cardiometabolic health. Furthermore, these data will support continued evaluation of BKR-017 for the regulation of weight and hypertriglyceridemia.

3. INVESTIGATIONAL PLAN SUMMARY

This study is an open-label study that will be conducted at Mayo Clinic. The total duration of subject involvement is approximately 11 weeks; 4-week Baseline Period which is followed by a 7 -week Test Period. During the Test Period, subjects will self-administer three tablets of investigational product (BKR-017) two times daily: before breakfast and before bedtime.

4. STUDY POPULATION

4.1 Number of Subjects

Approximately 28 subjects with T1D will be screened to determine eligibility. Sixteen eligible subjects will be enrolled into the study with the expectation that 13 will successfully complete the study.

4.2 Method of Assigning Subject Numbers

All subjects who consent to participate in the study will be assigned a unique three-digit number (assigned sequentially, in chronological order beginning with number 301). The subject number will be assigned at Visit 1 (Day -28) and will identify the subject for the remainder of the study.

4.3 Inclusion/Exclusion Criteria

Inclusion Criteria:

1. Males and females between the ages of 20 and 80 years at the time of screening, inclusive
2. Diagnosed with T1D and under the care of a healthcare professional for its management
3. HbA1c 6.4-8.9%, inclusive
4. Has given written informed consent to participate in this study
5. Willing to complete 28-day baseline period and 48 -day test period
6. Willing to maintain current diet and exercise routine for the duration of the study
7. Current use of a Dexcom Continuous Glucose Monitor (CGM)

Exclusion Criteria:

1. History of bariatric or intestinal surgery
2. Active gastrointestinal disease including but not limited to irritable bowel syndrome, inflammatory bowel disease (e.g., Crohn's disease and ulcerative colitis), diverticulitis, gastroparesis, or chronic/frequent diarrhea or chronic/frequent constipation

3. Active and clinically significant hepatic, pancreatic disease, or renal disease as determined by the investigator
4. History of significant heart disease, including congestive heart failure, prior MI, chronic atrial or ventricular fibrillation, coronary artery disease, cerebral vascular disease, or other cardiovascular disease, that in the opinion of the investigator should exclude the subject from the study
5. Severely uncontrolled hypertension at screening defined as a systolic blood pressure > 180 mmHg or a diastolic blood pressure > 110 mmHg on the average of two seated measurements after being at rest for at least 5 minutes
6. Uncontrolled hyperthyroidism or hypothyroidism, or other significant thyroid disease
7. Active significant infection as determined by the investigator
8. Known allergy to butyrate or any of the components of the tablets
9. Participation in a clinical trial and/or treatment with an investigational drug during the 30 days before screening, or within 5 half-lives of receipt of an investigational drug or twice the duration of the biological effect of any investigational drug (whichever is longer)
10. Pregnant, nursing, or trying to become pregnant
11. Presence of pitting edema on physical exam
12. High fiber diet
13. In the investigator's judgment, the subject is not suitable for the study for any other reason or cannot commit to the requirements of the study.
14. Subject is taking one or more of the excluded therapies. See list of excluded therapies in section 4.4.

4.4 Excluded Therapies

Subjects should not be enrolled in the study if they are currently (defined as 2 weeks prior to and at the screening visit) taking any of the following agents listed below:

1. GLP-1 receptor agonists (e.g., Byetta®, Bydureon®, Adlyxin®, Victoza®, Saxenda®, Trulicity®, Tanzeum®, Ozempic®)
2. Amylin analogs (e.g., Symlin®)
3. Thiazolidinediones (e.g., Actos®, Avandia®)
4. Any combination medications that contain 1-4 above (e.g., Avandamet®, Avandaryl®, Duetact®, Actoplus Met®, PioMet®)
5. Oral and injectable antibiotics
6. Oral or injectable corticosteroids (e.g., prednisone)
7. Chemotherapy
8. Opioids
9. Probiotics
10. Radiation
11. Cytotoxic therapy

12. Immune suppressants
13. Antiepileptic drugs
14. Colesevelam (Welchol®)
15. Acarbose (Glucobay®)
16. Miglitol (Glyset®)
17. Over-the-counter products that contain ephedrine
18. Other supplements containing butyrate
19. Medications that alter gastrointestinal motility including but not limited to metoclopramide (Reglan®), Metamucil® or other bulk fiber agents, emollient agents like ducosate, stimulants like Dulcolax® or castor oil, magnesium hydroxide (Milk of Magnesia®), hyperosmolar preparations (lactulose, sorbitol, MiraLAX®), opioids (e.g., Vicodin®, codeine, Percocet®), Adsorbents (Immodium®, Kaopectate®, Pepto-Bismol®), or anti-emetics (e.g., Zofran®, Kytril®, Compazine®, Phenergan®)

If a subject requires any of the above agents during the study, he/she should contact the study team to determine if there is an alternative available.

5. INVESTIGATIONAL PRODUCT

5.1 Investigational Product Description and Packaging

BKR-017 is a proprietary oral tablet formulation designed to target delivery of butyrate to the colon and provide sustained-release as the tablet passes through the colon. [redacted]

BioKier will arrange for the shipment of tablets directly from the manufacturer to the Mayo Clinic pharmacy in 150-ml HDPE white bottles (54 tablets per bottle). Each bottle contains enough test product for one week of dosing (plus 2 extra days). Each bottle will be labeled with the following information:

Study ID: CL-301 Investigator: Adrian Vella, MD
Mayo Clinic, Rochester, MN

Take 3 tablets before breakfast and 3 tablets before bedtime daily

Keep at room temperature

Investigational use only
Bottle contains 54 tablets of BKR-017

Subject Number: _____ **Bottle Number:** _____

5.2 Investigational Product Storage, Dispensing, and Return

Bottles containing test product (BKR-017 tablets) will be stored at room temperature at the Mayo Clinic Research Pharmacy.

The Research Pharmacist will keep detailed records of dispensation and return.

During Test Period

At Visit 1 (Day -28), eligible subjects will be given an 8-week supply of test product. Subjects will be instructed to begin dosing on Day 0. Additionally, Subjects will be instructed to complete the diary card daily and to return the diary card (with insulin usage, dosing information, changes in medications, and any adverse events) every 7 to 10 days by mail/email to the study team.

.

At Visit 2 (Day 48), the study CGM sensor, will be returned as well as all unused tablets in the original container(s). Diary cards will be reviewed and reconciled with the number of tablets returned. Subjects will be questioned about any missed doses based on the number of tablets returned, and the information supplied will be documented in the subject's study records.

5.3 Investigational Product Administration

The tablets will be self-administered orally twice daily; the first daily dose before breakfast, and the second daily dose before bedtime.

If the first daily dose is not administered before breakfast, it can be administered up to 12:00, noon. However, if the first daily dose is not taken by 12:00, noon it will be considered a missed dose; the bedtime dose should be taken as scheduled even if the morning dose was taken late or missed. If an evening dose is not taken by midnight, it will be considered a missed dose. All missed doses should be recorded on the diary card and returned in the original container at the next visit.

The study staff will counsel subjects who repeatedly miss doses about the importance of being compliant with the dosing regimen. However, if the investigator determines that a subject is not willing or able to reasonably comply with the dosing regimen, the subject should be discontinued.

5.4 Per Protocol Population

If a subject misses 15% of the required 96 doses (i.e., 14 or more doses) during the test period, then data from that subject will be excluded from the per-protocol efficacy assessment and included only in the intent-to-treat (ITT) analysis.

6. STUDY PROCEDURES

Table 1: Time & Events Schedule

	Pre-Screen	Baseline Period			Test Period			
		Visit 1						Visit 2
		D -28		D 0	D 18	D 28		D 48
Review of Eligibility & Study Requirements	X							
Written Informed Consent		X						
Review of Inclusion / Exclusion criteria		X						
Medical History & Demographics		X						
Blood Pressure (seated position at the start of visit)		X						X
Body Weight and Waist-to-Hip-Ratio		X						X
Hematology and Chemistry (including CPR)		X						X
Abbreviated Physical Exam		X						X
Urine Pregnancy Test (women of childbearing potential)		X						X
Dispense and/or Review Diaries for Compliance (Insulin and treatment)		X		X	X	X		X
Subject number assigned		X						
CGM sensors dispensed		X						
CGM sensor collected								X
Treatment Dispensed		X						
Fasting Glucose and Triglycerides		X						X
HbA1c		X						X
Exploratory Tests hs-CRP and 1,5AG		X						X
Concomitant Medication Review		X		X	X	X		X
Adverse Event Review				X	X	X		X

6.1 Screening Period

6.1.1 Recruitment and Prequalification

Subjects will be recruited through advertisements and other materials approved by the Mayo Clinic IRB. Potential study subjects interested in participating may also be referred to the research team.

Pre-screening will be performed to determine basic study eligibility information including:

- Age
- Type and age of onset of diabetes; confirmation of T1D diagnosis
- Understanding and willingness to commit to the study requirements
- Concomitant medications, including the need to refrain from use during the study of drugs that alter gastrointestinal motility

Subjects who meet the initial criteria and are willing to participate will be scheduled for a Screening Visit (Visit 1). Subjects will be required to fast (i.e., no food or beverages other than water) for at least 10 hours prior to the screening visit.

6.1.2 Visit 1 (Screening)

The screening visit will take place at the Outpatient Clinic (OPC), as described in Section 6.2.1 below, and include:

- Review of inclusion/exclusion criteria and eligibility
- Subject consent will be obtained; documented with an Informed Consent Form (ICF) signed by subject

6.2 Study Periods

The timing and evaluations for all visits to occur within the Baseline and Treatment Periods are defined in this section. Every attempt should be made to have all visits occurred at the required visit day. If, however, the subject is unable to adhere to the required visit day, a visit window of -/+ 2 days is allowed (i.e., 2 days prior or 2 days after the required visit day).

6.2.1 Baseline Period (Day -28 to Day 0, Visit 1)

The screening visit will take place on Day -28 before midday at the Outpatient Clinic (OPC). The following will be performed:

1. Review of inclusion/exclusion criteria and eligibility
2. Subject consent will be obtained; documented with an Informed Consent Form (ICF) signed by subject. Written informed consent must be obtained before any study procedures are

performed and a record of the subject's consent (i.e., an original, signed and dated consent form) is to be maintained in the study files and a copy provided to the subject.

Subjects whose HbA1c is within the range of 6.4-8.9% and meet all other preliminary eligibility criteria will continue with all other screening procedures, as follows:

1. Urine pregnancy test for females of childbearing potential (exclude if positive and do not continue with other screening assessments).
2. Confirm that the subject is in a fasted state.
3. Obtain blood samples for routine hematology and chemistry tests, including CPR for safety evaluations, plus fasting glucose and triglycerides. See Tables 3 and 4, Appendix 2, for safety laboratory evaluations.
4. Demographics (date of birth, gender, race, and ethnicity).
5. Confirm medical history, including HTN determination, and including diabetes classification, onset/diagnosis date of diabetes, diabetes therapy(s), and therapy duration(s).
6. Obtain concomitant therapies (generic name, dose, route, frequency, date started, and reason for use).
7. Obtain vital signs (heart rate and blood pressure) in a seated position.
8. Obtain subject's height and weight
9. Obtain waist and hip circumference to calculate the WHR.
10. Perform standard physical exam including an exam to detect any signs of edema. Any amount of pitting edema will exclude a subject.
11. After signing consent and meeting all inclusion criteria, subjects will be instructed on, and supplied with a study provided Dexcom G6 sensors, to collect glucose data. Subjects will be instructed to keep their Dexcom CLARITY app active, so that glucose monitoring data can be accessed by the study team.
12. Diary cards will be provided for the subject to record insulin doses, changes to concomitant medications and adverse events. Diary cards will be mailed/emailed to the study team every 7 to 10 days. Subjects using an insulin pump, will be advised to mail/email a copy of their pump settings and insulin doses every 7 to 10 days or upload their pump data to a prespecified, linked online account compatible with their pump (i.e. Medtronic CareLink, Tandem T Connect or Glooko), whereby the relevant data can be abstracted by the study team.
13. Subjects will receive an 8-week supply of test product (BKR-017)
14. Subjects will also be instructed not to make any modifications to their diet or exercise routine.

On Day 0:

1. Day 0 will occur approximately 28 days after the screen visit. The study team will communicate with the subject via a phone call or virtual visit on this day.
2. Study staff will review the subject's diary and CGM data, in order to confirm compliance.
3. Subjects will be questioned about any changes in health status/adverse events and any changes in therapy or medications.

4.

6.2.2 Termination of Baseline Period, Initiation of Test Period

Day 0, the following will be performed:

1. Subjects will be instructed to administer the first dose of test product prior to their mid-day meal and the second daily dose just prior to bedtime on Day 0.
2. Subjects will be instructed to keep their Dexcom CLARITY app active, so that glucose monitoring data can be accessed by the study team.
3. Subjects will be reminded to mail/email diary cards to the study team every 7 to 10 days. Subjects using an insulin pump, will be advised to mail/email a copy of their pump settings and insulin doses every 7 to 10 days or upload their pump data to a prespecified, linked online account compatible with their pump (i.e. Medtronic CareLink, Tandem T Connect or Glooko), whereby the relevant data can be abstracted by the study team.

4. Subjects will also be instructed not to make any modifications to their diet or exercise routine.
5. Subjects will be scheduled to return to the OPC for Visit 2 and instructed to return all unused tablets in the original container(s) along with any diary cards.

On Day 48 (Visit 2, Final Visit):

1. Subjects will report to the OPC in the morning, before 12:00 noon. If a subject is not able to comply with the Day 0 requirement, a variance of -2 days (or Day 46 or 50) is allowed.
2. Urine pregnancy test will be obtained for females of childbearing potential.
3. Subjects will return all unused tablets in the original container(s) along with any diaries.
4. Blood pressure, height, weight, and waist and hip circumference will be obtained to calculate the WHR. Fasting samples will be obtained for glucose and triglycerides. Additional blood samples will be obtained for HbA1c, routine hematology, routine chemistry assessments CPR, and for exploratory biomarkers hs-CRP and 1,5AG.
5. An abbreviated physical exam will be performed.
6. Study staff will review the subject's diary in order to reconcile tablets returned with any doses missed.

7. Subjects will be questioned about any changes in health status/adverse events and any changes in therapy or medications
8. Study personnel will remove the CGM sensor from the subject's abdomen and download the data to the Dexcom CLARITY data storage location.

7. SAFETY AND EFFICACY EVALUATIONS

7.1 Vital Sign Measurements

Single measurements of blood pressure and heart rate will be measured, with subjects in a seated position, at the start of every visit.

7.2 Tests for Safety Assessments

Hematology (CBC), and chemistry testing and CPR, will be performed at Screening (Visit 1) to determine eligibility and at the completion of the Test Period (Visit 2) to evaluate changes in lab values. The complete list of hematology and chemistry laboratory safety parameters to be collected is provided in Appendix 2; See Table 3 and Table 4.

A urine pregnancy test will be performed on women of child-bearing potential at screening to determine eligibility (pregnant women are not eligible) and at all subsequent Visits. Women who become pregnant at any time during the study should be discontinued at the investigator's discretion and all pregnancies need to be followed to document the outcome of the pregnancy.

In addition to the results of the urine pregnancy tests performed on women of child-bearing potential, study personnel will enter all laboratory data into the Electronic Case Report Form (eCRF).

Blood and urine samples will be collected, processed, and stored according to Mayo Clinic's established procedures.

All laboratory testing, except the 1,5-Anhydroglucitol (1,5-AG) samples will be tested at Mayo Clinic. The 1,5-AG samples will be tested by LabCorp.

All Mayo Clinic analytical testing will be performed by validated/established Mayo Clinic test methods or by methods routinely performed in the Mayo Clinic Core Laboratory.

Analytical testing performed at LabCorp will be performed by validated/established LabCorp test method.

7.3 Tests for Efficacy Assessments

7.3.1 Primary Efficacy Assessments

For the primary efficacy endpoint, HWR (Hip-to-Waist Ratio) and blood pressure will be measured at Day -28, Day 48 and used, along with HbA1c, to calculate the eGDR (estimated Glucose Disposal Rate) (WilliamsKV-ref 34).

7.3.2 Secondary and Exploratory Efficacy Assessments

Secondary and exploratory evaluations will also be performed. The specific evaluations and sample collection and analysis time points are listed in Table 3, Appendix 2.

Secondary efficacy evaluations require measurement of glucose variability, insulin usage, and triglycerides. Study personnel will enter the results into the eCRF.

For the secondary endpoint of glucose variability, Dexcom CGM sensors will be supplied by BioKier and used to record continuous glucose levels every 5 minutes during the study.

The CGM sensor is water-resistant and **must** be applied to the subject's abdomen. The CGM sensor is held in place with a self-adhesive pad and requires no subject interaction with the device or the need for calibration.

The CGM continuously measures glucose in interstitial fluid through a small (5mm long, 0.4mm wide) filament that is inserted just under the skin. It records glucose levels every 5 minutes, capturing up to 2880 glucose results for up to 10 days. Subjects will be instructed to keep their Dexcom CLARITY app active, so that glucose monitoring data can be accessed by the study team throughout the study. When the subject returns to the OPC at Visit 2, the CGM sensor will be removed by study staff and the data stored on the sensor will be retrieved by the reader and downloaded to the Dexcom CLARITY data storage location. The data will then be accessed by Mayo personnel and authorized BioKier personnel, including the statistician.

The other secondary efficacy endpoint, triglycerides, will be measured in blood samples from Visits 1, and 2 by the Mayo Clinic Laboratory.

Exploratory evaluations require analysis of the laboratory tests for hsCRP and 1,5-AG, from samples obtained Visits 1 and 2.

All blood samples for exploratory evaluations will be collected and will be processed according to Mayo Clinic's established procedures. All analytical testing will be performed on the secondary

and exploratory endpoints by validated/established Mayo Clinic test methods or by methods routinely performed in the Mayo Clinic Core Laboratory.

Study personnel will enter the exploratory test results into the eCRF.

7.4 Adverse Events

An adverse event is defined as any untoward medical occurrence that occurs during a clinical trial, whether or not considered related to the participation in the study or the test product received. Collection of adverse events in this study will begin at the time of administration of a subject's first dose of test product. Any untoward events that may occur between the screening visit and the first dose of test product should be recorded as medical history.

An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- Life-threatening
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- Congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the test product caused the adverse event. For the purposes of expedited safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the test product and the adverse event.

Subjects will be observed and questioned at every visit for the possible occurrence of adverse experiences; diaries will be reviewed to determine if any adverse experiences occurred between visits. Subjects should be asked to contact the investigator or study staff on any day on which an untoward event is experienced, whether the subject feels it is related to the test product or not.

All adverse experiences, whether observed by the investigator or study staff, or reported by the subject, will be recorded in the subject's study file and entered into the clinical database and will include the date of onset, outcome, date of resolution (if applicable), maximum severity, action taken, and relationship to the test product. Whenever possible, a diagnosis will be assigned rather than the constellation of symptoms.

Medical history/current medical conditions and adverse events will be coded using the medical dictionary (MedDRA) terminology.

8. PROVISIONS TO MONITOR DATA TO ENSURE SUBJECT SAFETY

The study protocol defines eligibility criteria designed to exclude patients for whom participation could pose unacceptable risks. Numerous medical assessments and laboratory tests throughout the study allow for continuous monitoring of safety. The Mayo Clinic Principal Investigator (PI) will review all lab work and adverse events. All adverse events will be reported to BioKier and the Mayo Clinic IRB in accordance with the Mayo Clinic IRB requirements.

9. DISCONTINUATION OF A STUDY PARTICIPANT

9.1 Screen Failures

A subject is considered a screen failure if he/she does not meet the inclusion/exclusion criteria or does not go on to receive test product for any reason.

9.2 Reasons for Discontinuation

A subject will be discontinued from the study for any of the following reasons:

1. Withdrawal of consent
2. Hospitalization
3. Treatment of an adverse event requires that the investigator be informed of the test product to which the subject was assigned
4. Death
5. Lost to follow-up
6. Sponsor or investigator requests discontinuation of a subject for safety reasons
7. Subject requires or has taken a prohibited medication for an extended period of time

8. Significant and/or persistent vomiting or diarrhea for an extended period of time, and vomiting/diarrhea can impact dosing/gut motility
9. Subject is non-compliant with any of the other requirements of the study

The reason for discontinuation will be documented in the subject's study record and relevant page of the eCRF.

For all subjects discontinuing before completing all study procedures and test visits, study personnel should perform a termination visit. At this visit subjects should return all unused test product and diary cards, vital signs should be obtained, and chemistry and hematology samples collected and tested. Additionally, if the subject is a woman of child-bearing potential, a urine pregnancy test should be performed.

If the subject is unable or unwilling to come to the clinical site for a termination visit, study personnel should conduct a follow-up telephone call to assess the reason for the subject's withdrawal from the study and to arrange for the return of unused test product.

For subjects lost to follow up, every effort should be made, including sending a certified letter, to contact the subject to come to the clinical site for termination visit procedures and to return test product.

10. DATA REVIEW AND DATABASE MANAGEMENT

10.1 Data Collection

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

Only specified study data and laboratory tests performed by Mayo Clinic will be entered into an eCRF. All data captured for this study must have an external originating source (either written or electronic); the eCRF is not considered as source.

The investigator must maintain source documents for each study subject, which consist of ICFs, case and visit notes (e.g., clinic medical records) containing demographic and medical information, laboratory data, diary card data, and the results of any other tests or assessments. All data must be traceable to these source documents. The investigator must also keep the original ICF signed and dated by the subject (a signed copy is given to the subject).

CGM data will be collected using the Dexcom G6 device. Data will be downloaded from the Dexcom G6 sensor to the Dexcom CLARITY data storage location in a manner that will allow accurate reporting and interpretation by the study team.

10.2 Database Management and Quality Control

The study database consists of data from the eCRF database, and data retrieved from the CGM sensors used during the study.

Study staff will enter required data into the eCRF on an ongoing basis as data are collected throughout the study. Automatic validation programs check for data discrepancies and generate appropriate error messages. Additionally, manual data queries will require resolution by the study personnel before query close-out. The eCRF will be accessible to authorized personnel only, and the system will be secured to prevent unauthorized access to the data and the system. This includes the requirement for a user ID and password to enter or change data. The level of access to the eCRF system (e.g., data entry, view rights only) will be dependent on each person's role in the study. The investigator will be required to certify (via electronic signature) that the data entered in the eCRF are complete and accurate.

Mayo Clinic personnel will review all data (eCRF) for completeness and accuracy and issue data queries about any inconsistent, incorrect or missing data. Once all queries have been resolved and the database is declared to be complete and accurate, it will be locked. After database lock, BioKier will provide the investigator with electronic media containing of all clinical study data for archiving.

10.3 Subject Confidentiality

In order to maintain subject confidentiality, only a subject number will be used to identify study subjects. Initials should not be used or referred to in any written communications.

The investigator is expected to comply with all applicable privacy regulations such as the Health Insurance Portability and Accountability Act (HIPAA) of 1996.

11. DATA ANALYSIS

Analysis of the data will be conducted under the direction of BioKier personnel in compliance with the Statistical Analysis Plan (SAP) and internal guidance documents and industry standards.

11.1 Objectives and Endpoints

11.1.1 Analysis Objectives

To evaluate the effects of BKR-017 tablets (active test product) when administered orally to T1D patients twice daily. Active test product will be administered for a 7 -week period.

11.1.2 Primary Efficacy Endpoint

The primary efficacy endpoint is the comparison of insulin sensitivity, calculated as the eGDR [34] on Days -28, and 48.

11.1.3 Secondary Efficacy Endpoints

Secondary endpoints include:

1. Glucose variability (from CGM data)
2. Fasting triglycerides
3. Insulin usage (from diary card)

11.1.4 Safety Endpoints

A descriptive assessment of safety will be made using vital signs, and selected chemistry assessments including bilirubin, alkaline phosphatase (ALP), asparate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine.

Additionally, clinically significant changes in the Visit 1 and 2 laboratory values obtained from routine hematology and chemistry (including CPR) testing will be reported in the eCRF along with adverse events reported during the test product administration period.

Adverse events reported during the study will be attributed to the test product administered at the time the event occurred.

11.1.5 Exploratory Endpoints

The exploratory endpoints include changes in measurements of hsCRP and 1,5-AG.

11.2 Sample Size

Differences in eGDR (mg/kg/min) between Days -28, and 48 will be assessed by calculating eGDR from HWR (Hip-to-Waist Ratio), blood pressure, and HbA1c, off treatment and on treatment. for each subject. Statistical significance will be assessed by comparing eGDR at each of Day -28and Day 48 to eGDR at Day -28 (i.e., baseline) with a paired t-test.

This study of 12 subjects is considered large enough to draw some preliminary clinical conclusions.

12. ANALYSIS CONVENTIONS

12.1 Analysis Sets

An analysis database of all efficacy data collected will be generated (ITT Population). In addition, an analysis set of all subjects without major protocol violations (per protocol population) will consist of all subjects who took at least 85% of the 96 required doses (i.e., 82 or more doses).

The safety analysis set will include all subjects who received any amount of test product.

Analysis plans are detailed further in a Statistical Analysis Plan document (SAP).

12.2 Missing Data

All missing data will remain missing. No values will be imputed.

12.3 Pharmacy Records

Mayo Clinic pharmacy study records will be made available to BioKier since BioKier is supplying the test product.

Inventory or dispensing records maintained by the Mayo Clinic pharmacy that identify the quantity of active test product that has been dispensed to a given subject will be reviewed.

12.4 Demographics and Baseline Characteristics

All data for background and demographic variables will be listed and summarized by subject. Relevant medical history, current medical conditions (including hypertension category (Y/N), results of laboratory screens, and any other relevant information will be listed. No statistical inference will be performed on these data.

12.5 Subject Disposition

The number and percentage of subjects in each analysis set who complete the study or discontinue early and the reasons for discontinuation will be summarized. No statistical testing will be performed.

12.6 Efficacy Analysis

All efficacy analyses will be repeated for both the ITT Population and the Per-Protocol Population.

12.6.1 Primary Efficacy Analysis

Differences in eGDR (mg/kg/min) between Days -28, and 48 will be assessed by calculating eGDR on Days -28, and 48 for each subject. Statistical significance will be assessed by separate within-subject comparisons of eGDR at each of Day -28, and Day 56 to eGDR at Day -28 (i.e., baseline) with paired t-tests.

12.6.2 Secondary Efficacy Analysis

Blood glucose variability will be calculated for each subject, for each 24-hour day (midnight to midnight) during which a CGM device recording is available for the entire 24-hour period. Results will be presented graphically for each subject by study day.

Triglyceride changes at Day -28 and Day 48 will be summarized as change from baseline where Day -28 is considered baseline.

12.6.3 Exploratory Efficacy Analysis

1,5 AG values at Day 48 will be compared to baseline (Day -28) with paired t-tests.

hs-CRP values at Day 48 will be compared to baseline (Day -28) with paired t-tests.

12.7 Safety Analysis

12.7.1 Adverse Events

The number and percentage of subjects experiencing adverse events reported during the test product administration period will be summarized by body system and preferred term for all subjects in the safety analysis set. Statistical testing will not be performed.

12.7.2 Laboratory Data

Selected laboratory tests will be summarized using an eight-number summary (sample size, mean, median, standard deviation, 25^{th} quartile, 75^{th} quartile, minimum, and maximum values) by visit. In addition, reported, graded laboratory abnormities will be summarized for each laboratory test. Statistical testing will not be performed.

12.7.3 Vital Signs

Vital signs will be summarized by visit using an eight-number summary (sample size, mean, median, standard deviation, 25^{th} quartile, 75^{th} quartile, minimum, and maximum values). Statistical testing will not be performed.

13. ETHICAL CONSIDERATIONS

13.1 Regulatory and Ethical Compliance

This clinical study was designed and will be implemented in accordance with the ethical principles established in the Declaration of Helsinki.

13.2 Informed Consent Procedures

Eligible subjects may only be included in the study after providing written (witnessed, if required) consent using an Institutional Review Board (IRB)-approved ICF. Informed consent must be obtained before conducting any study-specific procedures. The process of obtaining informed consent should be documented in the subject's medical record. Information should be given in both oral and written form and subjects must be given ample opportunity to inquire about details of the study.

13.3 Responsibilities of the Investigator and IRB

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted IRB before subjects can be enrolled in the study. For this study, Mayo Clinic IRB will be responsible for protecting the rights and welfare of human research subjects involved in the research activities and Mayo Clinic policies and procedures will be followed.

A signed and dated statement that the protocol and informed consent have been approved by the IRB must be provided to BioKier before study initiation. Prior to study start, the investigator is required to sign a protocol signature page confirming agreement to conduct the study in accordance with the protocol and to give access to all relevant data and records to BioKier representatives including study monitors, and Mayo Clinic IRB, as required.

13.4 Publication of Study Protocol and Results

Upon study completion and finalization of a study report, the results of this trial may be submitted for publication in accordance with the publication terms defined in the Clinical Study Agreement.

13.5 Protocol Adherence

Deviations from the protocol should be avoided. All protocol deviations will be documented, recorded in the eCRF, and reported in the study report. Deviations will be reported to the Clinic IRB in accordance with their requirements. No waivers will be granted by the study sponsor.

13.6 Protocol Amendments

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved the Mayo Clinic IRB. Only amendments that are required for subject safety may be implemented prior to Mayo Clinic IRB approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. In such cases, the Mayo Clinic IRB will be informed in conformance with the IRB requirements.

14. APPENDICES

14.1 APPENDIX 1: Previous Human Experience with Butyrate

Table 2: Previous human experience with butyrate (selected studies)

Patient Type	Stage	Dose/ Route of Administration	Duration	Reference
Oral and Colon-Targeted Formulations Type 2 Diabetes				
Type 2 diabetes	Efficacy, safety	1g/ rectal infusion	Single dose	BioKier Study [8]
Type 2 diabetes	Efficacy, safety	3g/day butyrate suppositories	4 weeks	BioKier Study (data on file)
Type 2 diabetes	Efficacy	6 x 100mg sodium butyrate capsules, orally	45 days	[35;36]
Oral and Colon-Targeted Formulations in Other Patient Populations				
Cancer, advanced solid tumors	Phase 1	50-400 mg/kg/d, oral	2 x 3 weeks	[37]
Cancer, advanced solid tumors	Pilot efficacy, safety	150-200 mg/kg TID, oral		[29]
Crohn's disease	Pilot efficacy, safety	4g/d, oral – enteric-coated tablets	8 weeks	[28]
Chronic pouchitis	Pilot efficacy, safety	4.4g/d, suppository	3 w	[27]

14.2 APPENDIX 2: Tables

Table 3: Safety Hematology Laboratory Tests

		Screening	Test Period
	Visit	1	2
	Day	- 28	48
White Blood Cell Count (WBC)		X	X
Red Blood Cell Count (RBC)		X	X
Platelet		X	X
Hemoglobin		X	X
Hematocrit		X	X
Mean Corpuscular Volume (MCV)		X	X
Mean Corpuscular Hemoglobin (MCH)		X	X
Mean Corpuscular Hemoglobin Concentration (MCHC)		X	X

Table 4: Safety and Efficacy Chemistry Laboratory Tests

ASSESSMENTS	Visit	Screening	Test Period					
		1						2
	Day	-28						48
Blood Urea Nitrogen (BUN)		X						X
Creatinine		X						X
Sodium, Calcium, Chloride, and Potassium		X						X
Carbon Dioxide		X						X
Total Protein		X						X
Albumin		X						X
Bilirubin (total)		X						X
Aspartate Aminotransferase (AST)		X						X
Alanine Aminotransferase (ALT)		X						X
Alkaline Phosphatase (ALP)		X						X
Cholesterol		X						X
Triglycerides		X						X
HDL		X						X
LDL		X						X
Glucose		X						X
CRP		X						X
HbA1C		X						X
Urine pregnancy test		X						X
Exploratory								
High-sensitivity C-reactive protein (hsCRP)		X						X
1,5-Anhydroglucitol (1,5-AG)		X						X

15. REFERENCES

(1) Drucker DJ. Biologic actions and therapeutic potential of the proglucagon-derived peptides. *Nat Clin Pract Endocrinol Metab* 2005;1:22-31.

(2) Toft-Nielsen MB, Damholt MB, Madsbad S et al. Determinants of the impaired secretion of glucagon-like peptide-1 in type 2 diabetic patients. *J Clin Endocrinol Metab* 2001;86:3717-3723.

(3) Ranganath LR, Beety JM, Morgan LM, Wright JW, Howland R, Marks V. Attenuated GLP-1 secretion in obesity: cause or consequence? *Gut* 1996;38:916-919.

(4) Thivolet C, Marchand L, Chikh K. Inappropriate glucagon and GLP-1 secretion in individuals with long-standing type 1 diabetes: effects of residual C-peptide. *Diabetologia* 2019;62:593-597.

(5) Liu L, Shao Z, Xia Y et al. Incretin-based therapies for patients with type 1 diabetes: a meta-analysis. *Endocr Connect* 2019.

(6) Plaisancie P, Dumoulin V, Chayvialle JA, Cuber JC. Luminal peptide YY-releasing factors in the isolated vascularly perfused rat colon. *J Endocrinol* 1996;151:421-429.

(7) Larraufie P, Martin-Gallausiaux C, Lapaque N et al. SCFAs strongly stimulate PYY production in human enteroendocrine cells. *Sci Rep* 2018;8:74.

(8) Szewczyk J, Giannone J, Marcuard S, Kindel T, Tso P, Nolan R. Colonic Delivery of Nutrients for Management of Blood Glucose in Type 2 Diabetes Patients. *The Journal ofFunctional Foods in Health and Disease* 2017;7:36-53.

(9) Gee JM, Johnson IT. Dietary lactitol fermentation increases circulating peptide YY and glucagon-like peptide-1 in rats and humans. *Nutrition* 2005;21:1036-1043.

(10) Weickert MO, Pfeiffer AF. Metabolic effects of dietary fiber consumption and prevention of diabetes. *J Nutr* 2008;138:439-442.

(11) Lu Y, Levin GV, Donner TW. Tagatose, a new antidiabetic and obesity control drug. *Diabetes Obes Metab* 2008;10:109-134.

(12) Gribble FM. RD Lawrence Lecture 2008: Targeting GLP-1 release as a potential strategy for the therapy of Type 2 diabetes. *Diabet Med* 2008;25:889-894.

(13) Ahren B. The future of incretin-based therapy: novel avenues--novel targets. *Diabetes Obes Metab* 2011;13 Suppl 1:158-166.

(14) Gribble FM, Meek CL, Reimann F. Targeted intestinal delivery of incretin secretagogues-towards new diabetes and obesity therapies. *Peptides* 2018;100:68-74.

(15) Jeppesen J, Hansen TW, Olsen MH et al. C-reactive protein, insulin resistance and risk of cardiovascular disease: a population-based study. *Eur J Cardiovasc Prev Rehabil* 2008;15:594-598.

(16) Melin EO, Thulesius HO, Hillman M, Landin-Olsson M, Thunander M. Abdominal obesity in type 1 diabetes associated with gender, cardiovascular risk factors and complications, and difficulties achieving treatment targets: a cross sectional study at a secondary care diabetes clinic. *BMC Obes* 2018;5:15.

(17) Buchwald H, Avidor Y, Braunwald E et al. Bariatric surgery: a systematic review and meta-analysis. *JAMA* 2004;292:1724-1737.

(18) Zhou J, Deng Z, Lu J et al. Differential therapeutic effects of nateglinide and acarbose on fasting and postprandial lipid profiles: a randomized trial. *Diabetes Technol Ther* 2015;17:229-234.

(19) Bunck MC, Corner A, Eliasson B et al. One-year treatment with exenatide vs. insulin glargine: effects on postprandial glycemia, lipid profiles, and oxidative stress. *Atherosclerosis* 2010;212:223-229.

(20) Tremblay AJ, Lamarche B, Kelly I et al. Effect of sitagliptin therapy on triglyceride-rich lipoprotein kinetics in patients with type 2 diabetes. *Diabetes Obes Metab* 2014;16:1223-1229.

(21) Xiao C, Dash S, Morgantini C, Adeli K, Lewis GF. Gut Peptides Are Novel Regulators of Intestinal Lipoprotein Secretion: Experimental and Pharmacological Manipulation of Lipoprotein Metabolism. *Diabetes* 2015;64:2310-2318.

(22) Dimitriadis G, Karaiskos C, Raptis S. Effects of prolonged (6 months) alpha-glucosidase inhibition on blood glucose control and insulin requirements in patients with insulin-dependent diabetes mellitus. *Horm Metab Res* 1986;18:253-255.

(23) Nagai E, Katsuno T, Miyagawa J et al. Effects of miglitol in combination with intensive insulin therapy on blood glucose control with special reference to incretin responses in type 1 diabetes mellitus. *Endocr J* 2011;58:869-877.

(24) Cani PD, Joly E, Horsmans Y, Delzenne NM. Oligofructose promotes satiety in healthy human: a pilot study. *Eur J Clin Nutr* 2006;60:567-572.

(25) Ibekwe VC, Khela MK, Evans DF, Basit AW. A new concept in colonic drug targeting: a combined pH-responsive and bacterially-triggered drug delivery technology. *Aliment Pharmacol Ther* 2008;28:911-916.

(26) Guidone C, Manco M, Valera-Mora E, Iaconelli A, Gniuli D, Mari A et al. Mechanisms of recovery from type 2 diabetes after malabsorptive bariatric surgery. Diabetes 55[7], 2025-2031. 2006.
Ref Type: Journal (Full)

(27) Wischmeyer P, Pemberton JH, Phillips SF. Chronic pouchitis after ileal pouch-anal anastomosis: responses to butyrate and glutamine suppositories in a pilot study. *Mayo Clin Proc* 1993;68:978-981.

(28) DiSabatino A., Morera R, Ciccocioppo R et al. Oral butyrate for mildly to moderately active Crohn's disease. *Aliment Pharmacol Ther* 2005;22:789-794.

(29) Edelman MJ, Bauer K, Khanwani S et al. Clinical and pharmacologic study of tributyrin: an oral butyrate prodrug. *Cancer Chemother Pharmacol* 2003;51:439-444.

(30) Weaver GA, Tangel CT, Krause JA et al. Acarbose enhances human colonic butyrate production. *J Nutr* 1997;127:717-723.

(31) Adrian TE, Gariballa S, Parekh KA et al. Rectal taurocholate increases L cell and insulin secretion, and decreases blood glucose and food intake in obese type 2 diabetic volunteers. *Diabetologia* 2012;55:2343-2347.

(32) Brunetti L, DeSantis EH. Patient tolerance and acceptance of colesevelam hydrochloride: focus on type-2 diabetes mellitus. *P T* 2015;40:62-67.

(33) Gadde KM, Vetter ML, Iqbal N, Hardy E, Ohman P. Efficacy and safety of autoinjected exenatide once-weekly suspension versus sitagliptin or placebo with metformin in patients with type 2 diabetes: The DURATION-NEO-2 randomized clinical study. *Diabetes Obes Metab* 2017.

(34) Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ. Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 2000;49:626-632.

(35) Roshanravan N, Mahdavi R, Alizadeh E et al. Effect of Butyrate and Inulin Supplementation on Glycemic Status, Lipid Profile and Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes: A Randomized Double-Blind, Placebo-Controlled Trial. *Horm Metab Res* 2017;49:886-891.

(36) Roshanravan N, Mahdavi R, Alizadeh E et al. The effects of sodium butyrate and inulin supplementation on angiotensin signaling pathway via promotion of Akkermansia muciniphila abundance in type 2 diabetes; A randomized, double-blind, placebo-controlled trial. *J Cardiovasc Thorac Res* 2017;9:183-190.

(37) Conley BA, Egorin MJ, Tait N et al. Phase I study of the orally administered butyrate prodrug, tributyrin, in patients with solid tumors. *Clin Cancer Res* 1998;4:629-634.