Effects of A3309, an Ileal Bile Acid Transport Inhibitor, on Gastrointestinal and Colonic Motor Functions in Female Patients with Functional Constipation

NCT# 01038687

June 7, 2010

Clinical Study Protocol	
Investigational Product	A3309
Study code	A3309-003
Protocol Version	1.3
Date	07 June 2010

STUDY TITLE	Effects of A3309, an Ileal Bile Acid Transport Inhibitor, on Gastrointestinal and Colonic Motor Functions in Female Patients with Functional Constipation

Development phase	II
Design	A single-center, randomized, parallel group, double-blind, placebo-controlled, dose response, pharmacodynamic and pharmacokinetic study.
Investigational product and dosage	Oral tablets of A3309, 15 or 20 mg once daily
Comparator product	Placebo
Duration of treatment	A3309 or matching placebo will be administered orally once daily for fourteen (14) consecutive days.
Sponsor Signatory	Hans Graffner, MD Albireo AB Arvid Wallgrens Backe 20 413 46 Göteborg, Sweden Telephone: +46 31 741 1480 email: hans.graffner@albireopharma.com
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Amendment No:1	**Date of Amendment :07 June 2010**
Amendment No:	**Date of Amendment :**

2 PROTOCOL SYNOPSIS

Study Title Effects of A3309, an Ileal Bile Acid Transport Inhibitor, on Gastrointestinal and Colonic Motor Functions in Patients with Functional Constipation	
Study code A3309-003	
Principal Investigator Michael Camilleri M.D. Professor of Medicine and Physiology, Division of Gastroenterology and Hepatology, Medical Director, Strategic Alliances, Mayo Clinic	
Study period Estimated date of first patient enrolled: January 2010 Estimated date of last patient completed: November 2010	**Phase of development** II
Study design This is a single-center, randomized, parallel group, double-blind, placebo-controlled, dose response, pharmacodynamic and pharmacokinetic study evaluating the effects of A3309 on gastric, intestinal and colonic transit in patients with functional constipation. Doses of 15 or 20 mg A3309 or matching placebo will be administered orally once daily for fourteen (14) consecutive days.	
Aim To assess the dose related effects of A3309 on small bowel and overall colonic transit and bowel function in patients with functional constipation. **Primary endpoint:** Colonic geometric center at 24 h as measured by scintigraphy, as compared to placebo. **Secondary endpoints:** 1. Colonic filling at 6 h measured by scintigraphy, as compared to placebo. 2. $T_{1/2}$ of ascending colon emptying measured by scintigraphy, as compared to placebo. 3. Colonic geometric center at 48 h measured by scintigraphy, as compared to placebo. 4. $T_{1/2}$ of gastric emptying of solid 5. Assess the effects on stool frequency and consistency based on the Bowel Pattern Diary and effects on symptom parameters coupled to constipation as well as the overall treatment effect, as compared to placebo based on a six question survey given at the end of the pre-treatment period and the post-treatment period. 6. Evaluate the pharmacokinetic (PK) and pharmacodynamic characteristics for each dose of A3309. 7. Assess the safety and tolerability of A3309 administered as an oral tablet.	
Number of patients planned Twelve female patients with functional constipation in each treatment group for a total of 36 patients.	
Diagnosis and main eligibility criteria Patients with diagnosed functional constipation will be recruited from the local community by public advertisement placed within areas of Mayo Clinic or by a targeted mailing of an informational letter.	

Methodology

Female patients with functional constipation will be screened for eligibility and informed about the study during pre-screening dialogue and also at the initial Visit 1 screen.

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

A urine pregnancy test will be performed for all females of child bearing potential at Visit 1 and again within the 48 hours prior to the receipt of radiation for both the abbreviated and post-study medication transit scintigraphy tests at Visit 2 and Visit 4. Note that females who are status post bilateral tubal ligation, hysterectomy or postmenopausal are exempted from this test.

Study medication will be administered at the Charlton 7 Clinical Research Unit (CRU) at Visit 4, 5, and 6, the days of scintigraphic assessment of gastric, small bowel and colonic transit of solids performed over a 48 hour period.

Within seven (7) to ten (10) days of Visit 6, patients will return to the Charlton 7 CRU for final safety monitoring and an exit physical examination and interview with study staff.

Investigational product, dosage and mode of administration

Patients will take 15 or 20 mg of A3309 or placebo administered orally for eleven (11) consecutive days and report for post-study medication transit scintigraphy on day twelve (12) of dosing. Study medication will be administered once with the In^{111} capsule on Visit 4 and once immediately before the camera images obtained on Visits 5 and 6. Study medication will be administered at Charlton 7 CRU by CRU nurses.

Duration of treatment

A3309 or matching placebo will be administered orally once daily for fourteen (14) consecutive days.

Duration of patients involvement in the study

Each patient will attend seven (7) visits at the clinic during a period of about thirty-one (31) to forty-one (41) days.

Efficacy assessments

- Scintigraphic small bowel and colonic transit
- Assessment of stool frequency and consistency using the Bowel Pattern Diary
- Symptoms of constipation
- Overall treatment effects

Pharmacokinetic and pharmacodynamic analysis

Blood samples for analysis of pharmacokinetic (PK) parameters will be collected at Visit 4, before dosing and at 30, 60, 90, 120, 180, 240, 360, 480 minutes post dosing. Pharmacodynamic parameters (C4, total cholesterol, HDL, LDL and triglycerides) will be collected at Visit 1, Visit 4 and Visit 7.

PK parameters will be analyzed using conventional analyses by Covance, UK

Safety assessments

The following safety assessments will be performed:

- Laboratory safety tests, including a complete blood count (CBC), a comprehensive metabolic panel (CMP), coagulation studies and a urinalysis (UA) performed at Visit 1 study entry, Visit 4 transit scintigraphy and Visit 7 study completion. A urine screen for drugs of abuse will be performed once at Visit 1 study entry.
- A 12-lead ECG performed at Visit 1 study entry, Visit 4 and Visit 7 study completion.
- A physical examination by a study physician at Visit 1 study entry, Visit 6 and Visit 7 study completion and as necessary throughout the study.
- Weight and vital signs (including temperature, pulse, blood pressure and respiration rate) at every visit
- Urine pregnancy tests performed at Visit 1 study entry and within 48 hours prior to receipt of radiation during the abbreviated transit scintigraphy and post-study medication transit scintigraphy at Visit 2 and Visit 4, respectively
- Interview for concomitant medications and adverse events at every visit

Statistical methods

An analysis of covariance (ANCOVA) will be used to compare transit parameters and patient bowel pattern diary summaries (ie, stool frequency, mean stool consistency and mean ease of passage scores) among the treatment groups. The covariates considered for inclusion in the analyses are age, body mass index (BMI) and the corresponding baseline (pre-treatment) bowel pattern diary summaries. If necessary a suitable transformation for potential skewness in the distributions of measured responses or heterogenous variation among treatment groups may be used (e.g., ANCOVA on ranks or square root, transformed stool frequency.)

If the ANCOVA test for overall treatment effects for a given response has a p value less than or equal to 0.10, then both the 15 mg and 20 mg doses will be compared to placebo using Dunnett's test.

Since each of the primary endpoints assesses a separate hypothesis regarding the effects of A3309, no adjustment in the alpha level for testing multiple types of endpoints is anticipated, and a two-sided significance level of 0.05 will be used in each ANCOVA model.

Statistical Power

Based on data acquired using the same methods in the laboratory, the sample size of 12 patients per group provides 80% power to detect differences of approximately 27% to 37% in colonic transit, the primary endpoints. This magnitude of change is considered clinically significant.

Pharmacokinetic and Pharmacodynamic analyses

Plasma concentration of A3309 *vs* time curves will be plotted for each subject, on both linear/linear and $\log_{10}$/linear scales. Mean plasma concentration *vs* time curves will also be presented by dose, PK and PD parameters.. Exploratory analyses of the PD variables will be conducted and described in the Statistical Analysis Plan (SAP).

Efficacy analyses

Exploratory analyses of the efficacy variables will be conducted and described in the Statistical Analysis Plan (SAP).

Analysis data sets

The primary analyses will follow the intent to treat (ITT) paradigm with all patients randomized included in the analyses. Those patients with missing response values will have their missing values imputed via the overall (patients with non-missing data) mean and a corresponding adjustment in the ANCOVA residual error variance degrees of freedom (subtracting one for each missing value imputed).

Safety data will be presented for all patients receiving investigational product.

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4 ABBREVIATIONS AND TERMS

Abbreviation/Term	Definition
A3309	Investigative drug substance code
A3309-^{14}C	Carbon-labeled drug substance
AE	Adverse event
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
AP	Alkaline phosphatase
aPPT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
BM	Bowel movement: A single defecation event with a beginning and end (as interpreted by the patient), which may include single or multiple stools.
BMI	Body mass index
bp	Blood pressure
C4	7α-hydroxy-4-cholesten-3-one
CIC	Chronic idiopathic constipation
C_{max}	Maximum plasma concentration observed post-dose
CRO	Contract research organization
CSBM	Complete spontaneous bowel movement: A spontaneous bowel movement that is accompanied by the patient reporting a feeling of complete evacuation.
CV	Cardiovascular
CYP_{450}	Cytochrome P_{450}
ECG	Electrocardiogram
GCP	Good Clinical Practice
GI	Gastrointestinal
HbA_{1c}	Glycosylated hemoglobin
IB	Investigator's Brochure
IBAT	Ileal bile acid transporter
IBS	Irritable bowel syndrome
IC_{50}	Concentration for 50% inhibition of the effect
ICF	Informed consent form

Abbreviation/Term	Definition
IRB	Institutional Review Board
ITT	Intent to treat analysis
i.v.	Intravenous
LDL-C	Low density lipoprotein cholesterol
NOAEL	No observed adverse effect level
NOEL	No observed effect level
PD	Pharmacodynamic
PK	Pharmacokinetic
SAE	Serious adverse event
SBM	Spontaneous bowel movement: A bowel movement that occurs in the absence of a laxative/enema use or manual disimpaction. For the purpose of this protocol, bowel movements that occur within 24 hours of laxative/enema use will not be considered spontaneous bowel movements.
SAP	Statistical Analysis Plan
$t_{½}$	Half-life
t_{max}	Time to maximum observed plasma concentration

5 ETHICS

5.1 Ethical review

Necessary approvals of the study protocol and the informed consent form (ICF) must be obtained before enrollment of any patient into the study. It is the responsibility of the Principal Investigator (PI) to keep the Institutional Review Board (IRB) informed of any Serious Adverse Events (SAEs) and any substantial amendments to the protocol during the study period.

The written approval from the IRB, including study identification and the date of review will be filed at the study site together with a list of the IRB members, their titles or occupation, and their institutional affiliations. All correspondence with the IRB should be filed both at the Sponsor and at the study site.

5.2 Ethical conduct of the study

The study will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects that were adopted in 1964 by the 18th World Medical Assembly, in Helsinki, Finland, with later revisions.

5.3 Patient information and consent

Informed consent will be obtained by the Investigator or study coordinator following both a review of the written ICF and a conversation outlining the risks, benefits, and goals of the investigation. The patient will be informed about the right to withdraw from the study at any time and should be allowed sufficient time for consideration of the proposal. Documentation will be recorded with signatures of the study participant and the individual obtaining consent (on the standard informed consent document) before any study-specific procedures are performed, including screening procedures.

The official Mayo IRB reviewed and approved version of the ICF must be used to obtain and document patient consent. The ICF must not be changed without permission from the sponsor Albireo AB and subsequent review and approval by Mayo IRB.

5.4 Patient data protection

The Investigator must file a patient identification list which includes sufficient information to link records, i.e. the Case Report Form (CRF) and the electronic medical record (EMR). This list will be preserved for possible future inspections/audits but should not be made available to the Sponsor except for monitoring or auditing purposes.

All data retrieved during the study will be used specifically for research purposes. Results will be maintained in Excel spreadsheets and SAS datasets stored electronically and will be password protected.

Mayo Clinic IRB guidelines for anonymizing data will be strictly adhered to. Only de-identified data will be permitted to be sent outside of Mayo Clinic. The ICF specifies that authorized representatives from the Sponsor, the IRB, FDA and other regulatory authorities will be granted access to the patient's medical record in a limited capacity for the purpose of verification of clinical study procedures.

5.5 Risks and Precautions

Data from two prior phase I studies using up to 5 mg in healthy volunteers and up to 10 mg in patients with functional constipation suggest that the study medication is well tolerated. In 26 normal healthy volunteers, the adverse events observed were: flatulence (27 percent), abdominal distension (19 percent), abdominal pain and nasopharyngitis (12 percent each), and diarrhea, nausea and headache, (2 percent each). In 25 constipation patients, the adverse events observed were: abdominal distension, nausea and headache (8 percent each). There was no relation of AEs to the dose levels tested. Further information is available in the Investigator´s Brochure Section 6.1

A Data Safety Monitoring Plan (DSMP) for this study has been established in accordance with recommendation by the Mayo IRB and CRU. We justify the proposal on the following rationale, that the study involves the use of radiation that is within limits permissible for patients with functional constipation. The study drug safety to date in healthy volunteers and patients with constipation has been excellent.

Radiation exposure in this study comes from the ^{99m}Tc and ^{111}In used to measure gut transit. These exposures conform to previously approved levels of radiation exposure approved by the Radiation Safety Committee at Mayo Clinic. The radiation dosimetry and organ exposures (in mrad) are listed below:

TRANSIT SCINTIGRAPHY

Radiopharmaceutical	Activity mCi	Body	Gonads	Breast	Red marrow	Lung	Thyroid
$^{111}InCl_3$	0.1	20	140		20		
^{99m}Tc sulfur	1.0	20	90		20		

Radiopharmaceutical	Bone	ULI	Colon	Stomach	Bladder	Liver	Esophagus	Other
$^{111}InCl_3$		380	740	60	40	10		160
^{99m}Tc sulfur		420	300	130	20	10		220

(mrad=radiation absorbed dose to organs)

H_e or the radiation effective dose to the body summarizes the risk to the whole body as the individual doses to each of the organs; effective dose is used to compare risks among various types of x-ray and radionuclide studies:

$^{111}InCl_3$	0.1 mCi	H_e 142 mrem
^{99m}Tc sulfur colloid	1.0 mCi	H_e 90 mrem

(mrem= radiation equivalent dose)

In summary, A3309 has a good safety profile, and the most commonly reported adverse events (AEs) in previous studies have been of gastrointestinal origin presumably reflecting the Mechanism of Action of A3309 and the subject cohort studied. The risks to the participants are reasonable since most procedures are noninvasive and there is no obvious risk of any significant morbidity or mortality.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

The study will be performed at the Division of Gastroenterology and Hepatology at the Mayo Clinic in Rochester, Minnesota. The study will be conducted under an Albireo sponsored IND, IND number 103,060 and the Investigational Product (IP) will be provided by Albireo.

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	Statistician/Co-investigator Alan R. Zinsmeister, PhD
Laboratory analysis	GI Physiology Core Laboratory at the Charlton 7 CRU, Mayo Clinic
Pharmacokinetic analysis	Covance Karolinska University Hospital SE-141 86 Huddinge, Sweden
Pharmacodynamic analysis	
Manufacturer of Active Pharmaceutical Ingredient (API)	Syngene International Ltd Biacon Park Plot no 2 &3 Bommasandra IV Phase Jigani Link Road Bangalore 560 099 INDIA
Packaging and labeling of API, IP quality control	Galenica AB Medeon Science park SE-205 12 Malmo, Sweden
Individual patient dose IP preparation, quality control and labeling	Mayo Research Pharmacy

6.1 Signature page

Protocol A3309-003

Title Effects of A3309, an Ileal Bile Acid Transport Inhibitor, on Gastrointestinal and Colonic Motor Functions in Female Patients with Functional Constipation

Reviewed by the following:

_____ _____

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Date

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Date

7 INTRODUCTION

7.1 Background

Constipation and C-IBS are very common clinical problems. A questionnaire study in Olmsted County, Minnesota demonstrated that up to 20% of patients report constipation (1) and up to 16% have some form of irritable bowel syndrome. Clinical practice suggests that patients transition between these two diagnoses, and it is also evident that the symptoms of these disorders overlap those of evacuation disorders such as pelvic floor dyssynergia. Thus, approximately 40% of patients with functional constipation have historical evidence of needing to strain excessively to pass bowel movements. These features might suggest that they also have a component of an evacuation disorder, although the questionnaire-based data did not allow a sufficient distinction between an evacuation problem and slow transit constipation. In a tertiary center study, 50% of 70 patients with severe, unresponsive constipation referred to a single gastroenterologist over a 3-year period had impaired evacuation, and the remainder of patients had either normal or slow transit constipation (2). Moreover, the cardinal "Manning" criterion of sense of incomplete rectal evacuation is conceivably a manifestation of a disturbance of rectal evacuation.

In clinical practice, it is essential to identify the presence of an evacuation disorder, particularly since the management of constipation should be modified by its presence (3). Biofeedback-based physical therapy is more effective than any other form of therapy when spastic pelvic floor disorders are identified. Therefore, patients with an evacuation disorder will be excluded from participating in this study.

A subgroup of patients (3,4) has functional or chronic idiopathic constipation (CIC), in which there is no evacuation disorder and colonic transit is slow or normal, but is not accelerated at baseline. This group of patients has a variable component of pain in association with constipation, and hence overlaps in time with C-IBS.

The cause of abnormal colonic transit in functional constipation, CIC and C-IBS is still incompletely understood; over the last three decades, histological studies have suggested that there may be quantitative differences in the number of interstitial cells of Cajal (ICCs), substance P (excitatory nerves reduced) and VIP or nitrergic (inhibitory nerves increased) neurons in the myenteric plexus of resected specimens of colons from patients with severe constipation. However, these results have not been consistently reported.

7.2 Current Approaches to Treatment of Constipation and C-IBS

Patients with constipation tend to benefit from fiber, osmotic laxatives (magnesium salts, PEG 3350 solutions), and stimulant laxatives (e.g., bisacodyl), although the efficacy of these in treatment of CIC, C-IBS and functional constipation is limited, even with recently approved medications such as lubiprostone. Prokinetics such as tegaserod have been withdrawn from the market. There is a spectrum of potential medications such as 5-HT4 agonists, and guanylate cyclase C agonists that are being tested but none are yet approved by the FDA. Given the fact that these conditions are not life-threatening, safety is of paramount importance and, hence, local activity within the gastrointestinal tract is beneficial in development of drugs for these indications.

7.3 Natural Laxation by Endogenous Bile Acids

Bile acids have a variety of physiologic functions and are actively reabsorbed (up to 95%) in the terminal ileum (5). Disruption of the enterohepatic circulation of bile acids due to ileal disease (e.g., Crohn's or

radiation ileitis) or idiopathic bile acid malabsorption (BAM) causes chronic diarrhea [see systematic summary (6)]. Conjugated and non-conjugated bile acids induce secretion in the human colon (7,8) by activating intracellular secretory mechanisms [e.g. adenylate cyclase (9)] or by detergent effects resulting in mucus secretion (10), increased mucosal permeability (11) and inhibition of apical Cl-/OH- exchange (12). In addition, bile acids may induce propulsive contractions in canine [>20mM (13)] and human colon [1mM (14) or 5mM (15)]. In humans, there is a relationship between the fecal bile acid excretion and colonic motility; however, this relationship is complicated by associated steatorrhea (15) since the delivery of fatty acids to the colon may also accelerate colonic transit (16). Bile acid concentration in stool of patients with ileal resection may reach 21mM chenodeoxycholate [CDC (17)]. However, fecal concentrations of bile acids in diarrhea-predominant irritable bowel syndrome (IBS-D) or functional diarrhea are unknown, and up-regulation of the ileal active transporter (18) as a result of chronic loss of bile acids may reduce the bile acids reaching the colon. In studies of gallstone dissolution with 750-1000mg CDC per day (19), diarrhea occurred in 40% of patients (20). Therefore, an approach that results in the passage of higher concentrations of endogenous bile acids into the colon has the potential to provide a useful treatment for constipation and related conditions. Structure activity studies show that several primary and secondary bile acids are secretagogues in the mammalian colon (11).

7.4 Safety of Delivering Higher Concentrations of Bile Acids to the Colon in Humans

The literature on gallstone dissolution shows that 37% of patients treated with typical CDC doses of 14-15 mg per kg per day had effectively dissolved gallstones (21) and had hypertransaminasemia (within 3 times upper limit of normal) in the first 6 months of treatment (22). On the other hand, doses of 7-20 mg CDC kg-1 day-1 were not hepatotoxic in humans (23). Among patients with gallstones treated with CDC (over 1000mg day-1 for several months), and patients who underwent partial ileal bypass for hyperlipidemia, there are no reports of colon cancer (24).

7.5 Unmet Clinical Need and Potential Indications for a A3309

Given the insufficient efficacy of OTC agents for CIC, FC and C-IBS, an approach that results in the delivery of endogenous bile acids to the colon is an attractive way to relieve constipation, especially with a locally acting agent that inhibits reuptake of bile acids by the apical bile acid transporter in the ileum. Such an agent is A3309. *From an academic perspective, this study also provides the opportunity to prove the principle that endogenous bile acids are effective laxatives to normalize bowel function, and also they provide proof of concept that bile acid malabsorption may be very relevant (and therefore targets for therapy) for patients with chronic diarrhea.*

8 DESCRIPTION OF A3309

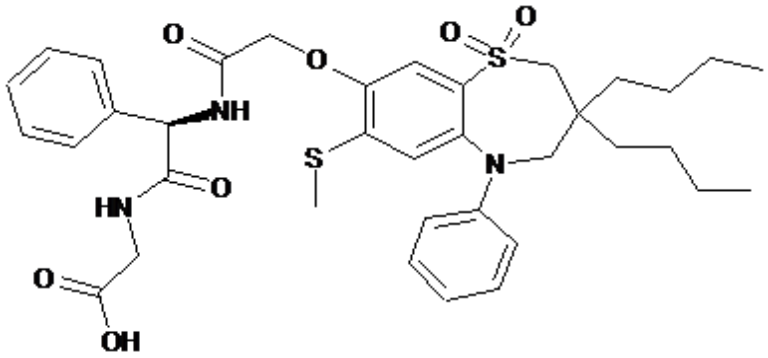
8.1 Chemistry

A3309 is a stereochemically pure enantiomer with a molecular weight of 695.9 g/mol. It is an acid with an acid dissociation constant (pKa) of approximately 4, the pH at which half of the molecules are ionized. As aqueous solubility is pH dependent, higher pH results in increased solubility.

The expected short half-life ($t_{½}$) of A3309 in humans is <4 hours.

The molecular formula of A3309 is $C_{36}H_{45}N_3O_7S_2$.

The chemical structure of A3309 is:



Bile acids are mainly absorbed by a specific ileal bile acid transporter in the ileum (IBAT) and are returned to the liver, completing enterohepatic circulation (25). A3309 is a small molecule known to be an inhibitor of the IBAT that interrupts the enterohepatic circulation of bile acids by decreasing intestinal bile acid reabsorption, which results in an increased bile acid load to the colon. This stimulates increased fluid secretion in the colon and colonic motility; both effects are expected to benefit patients with constipation (7). Additionally, given the mode of action of A3309, beneficial effects on bile acid synthesis and cholesterol levels are anticipated (25). Bile acid reabsorption from the intestine is a very efficient process whereby 95% of the secreted bile acids are reabsorbed and IBAT, an integral brush border membrane glycoprotein that co-transports sodium and bile acid, appears to be a major regulator of the bile acid pool in animals and man. IBAT inhibitors prevent the reabsorption of bile acids from the ileum and their return to the liver. The liver compensates for this decrease in bile acid level by upregulating cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis(26, 27).

8.2 Pharmacology

A3309 is a small molecule known to be an inhibitor of the ileal bile acid transporter (IBAT), [synonym: Apical sodium dependent bile acid transporter (ASBT)]. It has previously been evaluated for the treatment in dyslipidemia and is currently being evaluated for the treatment of constipation. The expected short half-life ($t_{½}$) of A3309 in humans is <4 hours.

Non-clinical studies

The species and strains of the animals used in the studies are presented in Table 1.

Table 1. The species and the strains used in the pharmacology, pharmacokinetic and toxicology evaluations of A3309

Species	Strain	Type of study
Rat	Wistar	Toxicology Safety Pharmacology
Rat	Long Evans black hooded	Quantitative whole body autoradiography (QWBA)
Dog	Beagle	Toxicology. Primary and Safety Pharmacology
Rabbit	New Zealand White	Reproductive toxicity

Species	Strain	Type of study
Mouse	CD1	Toxicology
Mouse	ApoE knock out	Pharmacology
Mouse	ApoE/LDL-receptor knock out	Pharmacology
Guinea pig	Duncan Hartley	Safety Pharmacology

Non-Clinical Pharmacology

Table 2. Primary pharmacology

Effect studied	Species (sex)	No of animals	Admin. route	Duration of treatment	Doses mg/kg (μmol/kg)
Effect on bile acid absorption	Mouse (F)	3	Oral	Single	0.11, 0.43, 1.7 (0.16, 0.63, 2.5)
Duration of action	Mouse(F)	3-4	Oral	Single	1.7 (2.5)
Effect on cholesterol lowering	Mouse (F)	7	Oral	7 days	1.7, 7 (2.5, 10)
Effect cholesterol metabolism	Rat (M&F)	3[a]	Oral	28 days	3.5, 35, 350 (5, 50, 500)
Effect cholesterol metabolism	Dog (M&F)	3[a]	Oral	28 days	3.5, 17, 140 (5, 25, 200)
Inhibition of bile acid uptake in HEK293 cells	Human, mouse and canine IBAT	3[b]	In vitro		
Effect on constipation	Dog (M&F)	28	Oral	3 days	1, 3.5, 10, 35 (1.5, 5, 15, 50)

[a] number of animals sampled at each time point at each dose level each day of exposure monitoring, for rats satellite groups were used (one month study)
[b] No. of experiments.
M=male F=female

A3309 is a potent inhibitor of the ileal bile acid transporter in human, mouse and canine IBAT-transfected cells as demonstrated by inhibition of bile acid uptake in this cell model (IC_{50}=0.53, 0.13 and 5.8 nM respectively).

Orally administered A3309 showed a dose-dependent inhibitory effect on the intestinal bile acid absorption in mouse with an ED_{50} value of 0.3 mg/kg. Moreover, A3309 showed a dose-dependent reduction (maximal effect 38%) of plasma cholesterol in the ApoE/LDLr KO mouse (transgenic mouse model showing a human-like lipoprotein profile). The cholesterol reduction was seen within the chylomicron/VLDL and LDL-fractions, whereas HDL-cholesterol fraction was increased.

The pharmacodynamic effect of A3309 was investigated in the one-month toxicity studies in rat and in dog by measuring the plasma concentration of 7α-hydroxy-4-cholesten-3-one (C4), a biomarker for bile acid synthesis. An increase of C4 in plasma was observed already after single doses in the dog. A dose dependent increase of C4 was observed in dog (3 to 7 times) and in rat (5 to 8 times) after the one month treatment period.

The effects of A3309 on GI motility and feces output were investigated in constipated beagle dogs. An increase in feces output was identified already at the lowest dose (1 mg/kg) tested and the magnitude of increase was similar to administration of tegaserod (0.3 mg/kg); a reference substance with known effects on GI motility in man. It was concluded that A3309 ameliorate meat-induced constipation in dogs.

The predicted therapeutic oral dose in man for A3309 is approximately 10 mg but, given scarcity of data, may be within the range of 5 to 20 mg, calculated from in vitro potency in human IBAT transfected cells and the in vitro and in vivo potency in mouse, dog and in man and from the results of the studies in healthy volunteers and in patients with chronic idiopathic constipation.

8.3 Safety Pharmacology

Table 3. Overview of safety pharmacology studies

Effect studied	Species (sex)	No. of animals	Admin. route	Duration of treatment	Doses mg/kg (μmol/kg)
Cardiovascular function	Guinea pig (M)	4	i.v.	Single	0.070, 0.41, 1.1, 2.5 (0.1, 0.6, 1.6, 3.6)
Motor coordination	Rat (M)	10	Oral	Single	3.5, 35, 350 (5, 50, 500)
Locomotor activity	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Irwin test, body temperature	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Hemodynamics and ECG	Dog (F)	6	i.v.	Bolus followed by infusion[a]	0.0035, 0.035, 0.35, 3.5 (0.005, 0.05, 0.5, 5)
Cardiovascular function	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Respiratory function	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Intestinal transit	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Renal function	Rat (M)	8	Oral	Single	3.5, 35, 350 (5, 50, 500)
Effect on potassium channel	hERG assay on human cells	6[b]	In vitro		0.70 (1)
Enzymatic and binding assays	Tissue models	3[b]	In vitro		0.070, 0.70, 7.0, 70 (0.1, 1, 10, 100)

[a] Cumulative dose during 30 min
[b] No. of experiments

Table 4. Plasma concentrations of A3309 (nmol/L). Compiled data from all safety pharmacology studies conducted in rat

Dose mg/kg (μmol/kg)	Sampling time post dose (hours)							
po	**1**	**1.5**	**2**	**3**	**4**	**6**	**24**	**30**
	(n=3)	**(n=8)**	**(n=3)**	**(n=6)**	**(n=11)**	**(n=9)**	**(n=32)**	**(n=8)**
3.5 (5)	26.0±3.0	40.6±19.5	20.7±5.6	12.2±6.1	6.8±2.1	4.0±1.9	nc	Nc
35 (50)	198±90	145±82	103±20	51.4±18.4	67.7±20.0	31.8±9.5	6.4±8.0	Nc
350 (500)	471±182	496±127	671±212	284±90	284±124	246±100	30±34	7.1±10.4

Nc = Not calculated, no mean was calculated if more than 40% of the concentrations were below LOQ. Mean values ± SD.

At the doses of A3309 studied in the rat, no evidence was found for any biologically relevant interaction with the central nervous system, respiratory function and there were no effects on arterial blood pressure, heart rate and body temperature observed. The only significant effect observed was on renal function (decrease in urinary excretion of sodium and increase in urinary concentration of urea) and on gastrointestinal motility (decreased) after the highest dose (350 mg/kg) given, which is more than 1000 times higher than predicted dose in man.

A3309 intravenously infused in the anesthetised dog at the doses of 0.0035, 0.035, 0.35, 3.5 mg/kg did not cause any pharmacologically relevant action on cardiovascular parameters. The only statistically significant change was a slight decrease in mean coronary blood flow and increase in coronary resistance. However, in the absence of any other hemodynamic effects, even at plasma concentration of 10,000 nmol/L, the effect on coronary flow is considered to be of no biological significance.

In addition, no cardiovascular effects were observed in the three-month toxicity study in dog at doses up to 140 mg/kg (520 times a human dose of 20 mg). A3309 had low effect *in vitro* in a variety of tissue models, enzyme/binding assay and on the hERG potassium channel; IC50 > 2000 fold higher than IC50 in the human IBAT transfected cell assay.

8.4 Pharmacodynamics in Animals

A3309 is an inhibitor of the ileal bile acid transporter in human, mouse and canine IBAT-transfected cells.

Orally administered A3309 showed a dose-dependent inhibitory effect on the intestinal bile acid absorption in mouse with an ED50 value of 0.3 mg/kg and a dose-dependent reduction (maximal effect 38%) of plasma cholesterol in a transgenic mouse model showing a human-like lipoprotein profile.

After oral administration of A3309, a dose dependent increase in plasma of 7α-hydroxy-4-cholesten-3-one (C4), a biomarker for bile acid synthesis, was observed in dog (3 to 7 times) and in rat (5 to 8 times).

In a study of the effects of A3309 on feces output in a dog model of meat-induced constipation, animals were dosed orally once daily for 3 days at 1.5, 5, 15, or 50 µmol/kg or with tegaserod (a compound previously used to treat constipation in humans) at dose levels of 0.3, 1.0, 66 µmol/kg. A3309 ameliorates meat-induced constipation in dogs already at the dose of 1.5 µmol/kg, with a statistically significant effect at a dose of 15µmol/kg. Tegaserod also reversed constipation in this dog model validating the method.The predicted therapeutic oral dose in man for A3309 is in the range of 5 mg to 20 mg with a predicted therapeutic target dose of approximately 10-15 mg/day, calculated from in vitro potency in human IBAT transfected cells and the in vitro and in vivo potency in mouse, dog, gastrointestinal effects on motility in healthy volunteers and in patients with chronic idiopathic constipation.

8.5 Pharmacokinetics and drug metabolism in animals

Orally administered A3309 appears to be minimally absorbed from the gastrointestinal tract. Following an oral dose of A3309-14C, most of the dose was found in the stomach and intestine and no radioactivity was detected in the blood in an autoradiography study in rats. The oral bioavailability in the dog was only

2%. Cmax is generally reached within 2 hours after oral administration to rat and dog and is eliminated from plasma with a half-life < 2 hours.

A3309 is highly bound to plasma proteins, >99.5%. The tissue distribution of A3309-14C was limited after an oral dose, and the radioactivity was rapidly eliminated; 24 hours after dose, compound related material was only seen in the gastrointestinal tract.

Upon repeated oral dosing of A3309 there is no evidence of time dependence or accumulation in either rat or dog. The exposure increased proportional with dose except at the highest doses in the toxicity studies, where the exposure increased more than dose proportional in the rat (AUC) while less than dose proportional in the dog.

A3309 does not inhibit heterologously expressed human CYP1A2, CYP2C19 and CYP2D6 up to 20 µM. It inhibits CYP2C9 and CYP3A4 exhibiting IC50 values of 10.3 and 6.0 µM respectively. That the absorption of A3309 is low indicates a low risk of drug-drug interactions with these enzymes after oral dosing.

8.6 Toxicology and Safety Pharmacology

At the doses of A3309 in the safety studies (3.5-350 mg/kg) in the rat, no evidence was found for any biologically relevant effect on central nervous system and respiratory function and there were no effects on arterial blood pressure, heart rate or body temperature. The only significant effect observed was on renal function (decrease in urinary excretion of sodium and increase in urinary concentration of urea) and on gastrointestinal motility (decreased) after the highest dose (350 mg/kg) given, which is more than 1000 times higher than a clinical dose of 20 mg. A3309 intravenously infused in the anesthetised dog at the doses of 0.0035, 0.035, 0.35 and 3.5 mg/kg did not cause any pharmacologically relevant action on cardiovascular parameters. The only statistically significant change was a slight decrease in mean coronary blood flow and increase in coronary resistance. However, in the absence of any other hemodynamic effects, even at plasma concentration of 10,000 nmol/L, the effect on coronary flow is considered to be of no biological significance.

The acute toxicity of A3309 is considered to be low after oral administration. The minimum lethal dose after oral administration in rats and mice is >2000 mg/kg. Dogs were given single oral ascending doses of A3309 at 8.4, 17, 35, 70 and 140 mg/kg. The animals were necropsied 14 days after completion of dosing. Clinical signs observed after treatment were loose stool, diarrhea and emesis. The frequency and severity increased with dose starting at 17 mg/kg. There were no histopathology changes that could be attributed to A3309.

In a preliminary rat study, enteritis of the caecum was observed in most animals dosed at 200 or 1000 mg/kg for 7 days. However, the toxicological significance of this finding is unclear since rats dosed with up to 350 mg/kg/day of A3309 for one or three months showed no effects in the gastrointestinal tract.

In both the one- and the three-month toxicity study in rats, groups of 10 males and 10 females were given A3309 orally once a day at 0, 3.5, 35 and 350 mg/kg for 28 days and 13 weeks respectively. None of the changes seen in A3309-treated rats were considered to be of toxicological significance. NOAEL (no observable adverse effect level) in rats was at least 350 mg/kg/day in both studies.

In a one-month toxicity study in dogs, groups of 3 males and 3 females were given A3309 orally once daily for 28 days at 0, 3.5, 17 and 140 mg/kg/day. The only blood chemistry change considered

noteworthy at these dose levels was a slightly reduced level of plasma triglycerides. For all other parameters, including histopathology, there were no toxicologically significant, treatment-related changes. Based on the findings of emesis and soft/fluid feces at 17 and 140 mg/kg the NOAEL in dogs was considered to be 3.5 mg/kg/day. However, excluding these signs of mild gastrointestinal intolerance there were no effects of toxicological significance at doses up to and including 140 mg/kg.

In a three-month toxicity study in dogs, groups of 4 males and 4 females were given A3309 orally once daily for 13 weeks at 0, 3.5, 17.5 and 140 mg/kg/day. Dose related liquid feces were seen in all groups treated with A3309. An increase in liver weight and minor changes in red blood cell parameters at high dose was not considered toxicologically significant. NOAEL for males was 140 mg/kg/day but in females 17.5 mg/kg/day based upon emesis occurring at the higher dose.

Thus, the main target organ for A3309 in the toxicology studies appears to be the gastrointestinal tract. It is most likely that the effects seen in the gastrointestinal tract are related to the pharmacological activity of A3309, e.g. increased colon motility. Based on the available toxicological documentation, it is concluded that A3309 can be given orally to man up to three months of duration in the proposed clinical trials with sufficient safety.

8.7 Reproductive Toxicology

No adverse effects on fertility or embryo-fetal development were seen in the repro-toxicity studies in rat and rabbit. The doses of A3309 used exceeded a clinical dose of 20 mg by about 600- 4000 fold. In a fertility and embryo-fetal study in rats, groups of 22 males and 22 female rats were given A3309 at 0, 175, 350 or 1000 mg/kg/day for 2 weeks prior pairing and continued until day 17 (females) and day 20 (males) of gestation. The NOAEL was 1000 mg/kg/day. In an embryo-fetal study in rabbits, groups of 24 mated females were given daily oral doses of A3309 at 0, 20, 55 and 150 mg/kg/day from day 7 to day 19 of gestation. The NOEL was 150 mg/kg/day.

8.8 Effects in Humans

There have been two main studies to date in humans: a single and repeated-dose, single-blind, randomized, placebo-controlled, Phase I study to assess the tolerability, safety and pharmacokinetics of A3309 after single escalating oral doses and repeated doses in healthy male subjects (D1240C00001)

1. The single doses were 0.1, 0.5, 2.5 and 5 mg. The repeated dose was 0.25 mg daily for seven days. In total, 38 subjects were randomized; thirty subjects to single doses of A3309 or placebo and eight subjects to repeated doses or placebo. Tolerability was monitored by global physical examination, 12-lead ECGs, safety laboratory assessments and recording of adverse events. In addition, specific evaluation of gastrointestinal symptoms (Gastrointestinal Symptom Rating Scale – GSRS), bowel movement frequency and stool consistency were evaluated. Pharmacodynamic markers of dyslipidemia and bile acid synthesis evaluated were campesterol/sitosterol, lanosterol and 7α-hydroxy-4-cholesten-3-one (C4). No effect on lipid profiles was noted. However, this is not expected after a single dose. There was an effect on C4 in the 2.5 and 5 mg single dose cohorts. Pharmacokinetic samples were scheduled throughout the 24 hour observation period in the single dose groups. No measurable plasma or urine concentrations of A3309 were detected; neither in the single ascending dose part (up to and including 5 mg) or in the multiple dose group (0.25 mg daily for seven days). In 26 normal healthy volunteers, the adverse events observed were: flatulence (27 percent), abdominal distension (19 percent), abdominal pain (12 percent), and diarrhea, nausea and headache, (2 percent each).Adverse events, other than gastrointestinal problems, were few and in most cases mild in intensity and did not cause any reason for concern. One 25 year old male developed bloating, passage of mucus and had proctitis on colonoscopy 6 weeks later. There were no clinically relevant changes in clinical chemistry, hematology, coagulation, vital signs, weight, ECG, physical observations, GSRS, or stool appearance during the study.

2. A double-blind, randomized, placebo-controlled, phase Ib, dose-escalating study to assess the safety, tolerability and systemic exposure of A3309 capsules after repeated once daily administration to patients with chronic idiopathic constipation (Study A3309-001). Eligible patients should have had at least a 12 month history of constipation (defined as < 3 BMs per week) with symptoms and no more than 5 spontaneous complete bowel movements (SCBMs) during the 14 days preceding randomization. The initial screening evaluation included flexible sigmoidoscopy, colon transit measurement and a 14 day period to evaluate frequency of BMs and to calculate baseline data for GI symptoms. The primary objective of this study was to assess the safety and tolerability of A3309 after repeated oral doses to patients with chronic idiopathic constipation (CIC). Secondary objectives of the study were to assess: 1/ changes in (SCBM) and Bristol Stool Form score after repeated oral doses of A3309, 2/ to investigate colon transit and systemic availability of A3309 during escalating oral repeated doses and 3/ to evaluate the effect of A3309 by analysis of plasma glucose or HbA1c, triglycerides (TG), plasma lipid parameters, FGF19, FGF21 and C4. Patients were randomized in a 5:1 ratio to receive one of five sequential dose levels (0.1 mg, 0.3 mg, 1.0 mg, 3.0 mg or 10 mg) of A3309 or placebo, orally administered. A blinded safety assessment was performed between each dose level. The safety evaluation do not indicate any apparent effects on physical examination findings, vital signs, safety laboratory or 12-lead ECGs and no serious adverse events were reported. In 25 constipation patients, the adverse events observed were: abdominal distension, nausea and headache (8 percent each). The small number of subjects and events do not permit conclusions about any difference between groups in the nature, severity or incidence of adverse events. No deaths were reported and no adverse events led to treatment or study discontinuation. Pharmacokinetics: A3309 was detected in plasma in the picomolar range at doses of 1-10 mg. The highest individual levels at 10 mg were 0.33-0.76 nmol/L, seen 2 hours after 14 days of treatment. Cmax occurs within 4 h after dose. There is no obvious accumulation of neither A3309 or any metabolite since there were no detectable levels 24 hours after dose. The pharmacodynamic analyses provided evidence of biological activity consistent with the mechanism of action of A3309:

- C4, a biomarker of bile acid synthesis, showed a dose-dependent increase from baseline to end of treatment (p=0.0042). Values returned to baseline at the follow-up 30 days after end of treatment.
- Total cholesterol showed a dose-dependent decrease from baseline to end of treatment (p=0.0305) and a similar trend was observed for LDL cholesterol (p=0.0536).
- This study was not powered to show an effect on clinical endpoints, or a comparison to placebo.

However, pre-planned as exploratory analyses, the following parameters indicate therapeutic effect with A3309:

- Colon transit in Days comparing baseline with an evaluation on last day of treatment; p=0.0129 (test for trend over increasing dose)
- Change from Baseline to Week 1 for number of spontaneous bowel movements (SBMs); p= 0.0285 (test for trend over increasing dose)
- Most of the observed signals of clinical effect were identified in the 10 mg group.

The overall conclusions from the dose levels tested:

- **Very low systemic exposure** (picomolar range in highest dose groups) was observed.
- Administration of A3309 was found to be **safe and tolerable** across the dose levels tested with no serious adverse events or discontinuations.
- Clear **biological effect** on C4, total cholesterol and LDL cholesterol and colon transit.
- Preliminary descriptive evidence of clinical efficacy: bowel movement frequency.

9 STUDY AIMS AND ENDPOINTS

9.1 Aim

To assess the dose related effects of A3309 on small bowel and overall colonic transit and bowel function in patients with functional constipation.

9.2 Primary endpoint:

Colonic geometric center at 24 h as measured by scintigraphy, as compared to placebo.

9.3 Secondary endpoints:

1. Colonic filling at 6 h measured by scintigraphy, as compared to placebo.
2. $T_{1/2}$ of ascending colon emptying measured by scintigraphy, as compared to placebo.
3. Colonic geometric center at 48 h measured by scintigraphy, as compared to placebo.
4. $T_{1/2}$ of gastric emptying of solid
5. Assess the effects on stool frequency and consistency based on the Bowel Pattern Diary and effects on symptom parameters coupled to constipation as well as the overall treatment effect, as compared to placebo based on a six question survey given at the end of the pre-treatment period and the post-treatment period.
6. Evaluate the pharmacokinetic (PK) characteristics and pharmacodynamic effects for each dose A3309.
7. Symptoms of constipation

10 RESEARCH DESIGN AND METHODS

This is a randomized, single-center, parallel-group, dose-response, multiple-administration, double-blind, placebo-controlled study evaluating the effects of A3309 on gastrointestinal and colonic transit in patients with functional constipation. Following an initial screening (visit 1), patients will have baseline transit measurement over 24 hours to ensure they fulfill entry criteria for transit. Subsequently, they will be randomized to one dose of A3309 or placebo per day for 14 days, and will undergo scintigraphic assessment of gastric, small bowel and colonic transit of solids starting on day 12. These studies will be undertaken on over a 48 hour period, days 12 to 14, (visits 4-6). Participants will undergo a safety follow-up visit (visit 7) 7-10 days after the last day of study medication. Three treatment arms will be included in the study. As stated above, participants will be randomly assigned and allocation will be concealed.

11 OVERALL STUDY DESIGN AND SCHEDULE OF EVENTS

11.1 Study Design

This is a single-center, randomized, parallel group, double-blind, placebo-controlled, dose response, pharmacodynamic and pharmacokinetic study to evaluate the effects A3309 on gastrointestinal and colonic transit in patients with functional constipation. Patients with functional constipation will be screened for eligibility and informed about the study during pre-screening dialogue and also at the initial Visit 1 screen.

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

A urine pregnancy test will be performed for all females of child bearing potential at Visit 1 and again within the 48 hours prior to the receipt of radiation for both the abbreviated and post-study medication transit scintigraphy tests at Visit 2 and Visit 4. Note that females who are status post bilateral tubal ligation, hysterectomy or postmenopausal are exempted from this test.

Study medication will be administered at the Charlton 7 Clinical Research Unit (CRU) at Visit 4, 5, and 6, the days of scintigraphic assessment of gastric, small bowel and colonic transit of solids performed over a 48 hour period.

Within seven (7) to ten (10) days of Visit 6, patients will return to the Charlton 7 CRU for final safety monitoring and an exit physical examination and interview with study staff.

11.2 Schedule of events

Study assessments are described in the chart below.

Table 5 Study assessments.

	V1	V2	V3	V4-V6	V7
Event	**Screen**	**Baseline Transit (V1 + 7-14 days)**	**Randomization (V2 + 0-3 days)**	**Final Transit (days 12-14 of study med)**	**Follow-up (V6 + 7-10 days)**
Patient information	x				
Informed consent	x				
Inclusion/exclusion criteria	x				
Demographics	x				
Medical history	x				
Physical examination[1]	x			x	x
Height/Weight	x				x
Vital signs[2]	x	x	x	x	x
Med history/adverse events[3]	x	x	x	x	x
Concomitant medication	x	x	x	x	x
Laboratory safety tests[4]	x			x	x
Pharmacodynamics[5]	x			x	x
Pregnancy test[6]	x	x		x	
12-lead ECG	x			x	x

	V1	V2	V3	V4-V6	V7
Event	**Screen**	**Baseline Transit (V1 + 7-14 days)**	**Randomization (V2 + 0-3 days)**	**Final Transit (days 12-14 of study med)**	**Follow-up (V6 + 7-10 days)**
Long BDQ	x				
Bowel pattern diary	x (out)				X (in)
Symptom and efficacy survey[7]			x	x	
Scintigraphy[8]		x		x	
Randomization[9]			x		
Pharmacokinetics[10]	x			x	x
Administration of study med			x	x	

[1] A rectal exam to be performed at Visit 1 to exclude the possibility of an evacuation disorder if a normal exam not documented within 2 years by a qualified gastroenterologist. A patient may defer the rectal exam at Visit 6 and 7 if not clinically indicated..
[2] Includes temperature, blood pressure, pulse and respiratory rate
[3] Adverse events occurring prior to administration of study medication are classified as medical history
[4] Laboratory safety assessments include clinical chemistry (ALT; Albumin; Alkaline phosphatase; AST; Bilirubin, total; Calcium; Chloride; C-Reactive Protein; Creatinine; GGT; Glucose; Potassium; Protein, total; Sodium; Urea; Uric acid), hematology (hemoglobin; hematocrit; MCV; platelet count; RBC count; WBC count with differential), coagulation studies (Activated partial prothrombin time aPPT; Prothrombin time PT), urinalysis (blood; glucose; ketones; leukocytes; nitrites; pH; protein), drugs of abuse urine screen (alcohol; amphetamines; barbiturates; benzodiazepines; cocaine; opiates; phencyclidine; tetrohydrocannibinol). The drugs of abuse urine screen is performed once at Visit 1; the other laboratory tests are performed at Visit 1, Visit 6 and Visit 7.
[5] Blood samples for pharmacodynamics including C4 and a lipid panel of total cholesterol, HDL, LDL and triglycerides are obtained at Visits 1, 4 and 7.
[6]Females of child bearing potential are required to have a negative pregnancy test result within 48 hours prior to the receipt of radiation. Females who are status post-tubal ligation, post-hysterectomy or post-menopause are exempted from this requirement
[7] A six question survey concerning constipation symptoms and treatment efficacy will be given at Visit 3, the end of the pre-treatment period, and again at Visit 6, the end of the treatment period.
[8]An abbreviated transit scintigraphy with scans obtained at 4 and 24h is performed at Visit 2. A full transit with scans obtained at 0, 30 min, 60 min, 90 min, 2, 3, 4, 5, 6, 7 and 8 hours after taking study medication for 12 consecutive days at Visits 4-6.
[9]Criteria for randomization is defined as a geometric center < 2.30 at 24h, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies)
[10]Blood samples for pharmacokinetics are obtained at Visit 4 at pre-dose, 30, 60, 90, 120, 180, 240, 360, 480 minutes after study medication. One blood sample for pharmacokinetics is obtained at Visit 1 and Visit 7 as well.

11.3 Schedule of Visits

Visit 1 Study Entry
Potential study patients will arrive at the Charlton 7 CRU fasting for the previous eight hours. Patients will be required to sign the ICF after review and explanation of the study and after all questions have been answered. Inclusion and exclusion criteria will be accurately reviewed.

After reviewing and signing a patient ICF, they will have a brief interview (including demographics, medical history and current medications) and a physical examination performed by a study physician. If the results of a normal rectal examination to exclude the possibility of an evacuation disorder are not documented within the previous two years by a qualified gastroenterologist, this exam will be performed

at this time. Height, weight and vital signs including temperature, pulse, blood pressure, and respiration rate will be obtained.

Blood and urine samples will be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies, UA, urine screen for drugs of abuse and a urine pregnancy test for females of childbearing potential. Note that females who are status post bilateral tubal ligation, hysterectomy or postmenopausal are exempted from this test. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total amount of blood obtained for the standard laboratory tests is about 30 mL. A 12-lead ECG will be performed.

Patients will review the study instructions and schedule with the study coordinator. The Long Bowel Disease Questionnaire (BDQ) will be completed to confirm patients have functional constipation. The Bowel Pattern Diary will be dispensed and reviewed.

Prior to Visit 2
Females of child bearing potential must have a negative urine pregnancy test performed within forty-eight hours prior to receipt of radiation in the abbreviated transit scintigraphy test. Females who have experienced a bilateral tubal ligation, hysterectomy or menopause will not need the pregnancy test.

Visit 2 Abbreviated Transit Scintigraphy
Within 7-14 days of Visit 1, patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. They will be given a capsule containing a small amount of In^{111} radioactivity and asked not to eat anything for the next two hours and to return four hours later for one five minute camera picture. Patients will return the following morning at the same time the capsule was taken for one final five minute camera image. If the camera indicates a state of constipation by showing a geometric center at 24 hours to be less than 2.30, , the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies ,the patient will continue in the study and be randomized to the study medication. If the camera does not indicate a state of constipation by showing a geometric center equal to or greater than 2.30, the patient will end the study at this time.

Visit 3 Randomization and Administration of Study Medication
The patient will complete a six question survey of constipation symptoms and treatment efficacy at the end of the pre-treatment period, before receiving study medication. The patient will then be randomized to a dose of the study medication which is either a placebo (an inactive substance) or a 15 mg or 20 mg dose of A3309 study medication taken orally once daily for a total of fourteen consecutive days, eleven doses at home and three at the Charlton CRU during the final transit scintigraphy test, Visit 4 to Visit 6. Patients will dose at home and record the dosing of study medication in their Bowel Pattern Diaries.

Prior to Visit 4
Females of child bearing potential must have a negative urine pregnancy test performed within forty-eight hours prior to receipt of radiation in the abbreviated transit scintigraphy test. Females who have experienced a bilateral tubal ligation, hysterectomy or menopause will not need the pregnancy test.

Visit 4 Final Transit Scintigraphy and Pharmacokinetic Sampling
Patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses.
The study medication and a capsule containing a small amount of In^{111} radioactivity will be given. About one hour after taking the capsule, the first anterior and posterior images will be obtained. The patient will be given a scrambled egg breakfast meal; the eggs contain a small amount of Tc^{99} radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging

procedure or the 'zero hour' image. Images will be obtained at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line will be placed in the forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis. Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360, 480 minutes after study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies, and UA. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about 75 mL. A 12-lead ECG will be performed. About four hours after the breakfast, a standard chicken breast lunch meal is given. Images will be obtained at five, six and seven hours. About eight hours after the breakfast, a standard roast beef sandwich snack, will be given followed by a final camera image. These standardized meals ensure that all participants in this study eat the same type and same amount of food this day. Patients are asked not to eat or drink anything other than these standardized meals.

Visit 5 Transit Scintigraphy
The following morning**,** patients will return to the Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed, to receive the study medication followed by one five minute camera image. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses.

Visit 6 Transit Scintigraphy
The following morning**,** patients will return to the Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed, to receive the study medication followed by one five minute camera image. A brief physical exam will be performed by a study physician. Patients may defer the rectal exam if not clinically indicated. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses. Patients will complete a six question survey of constipation symptoms and treatment efficacy.

Visit 7 Study Completion
Within seven to ten days after Visit 6, patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Blood and urine samples will be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies and urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total amount of blood obtained for the standard laboratory tests is about 30 mL. A 12-lead ECG will be performed. The Bowel Pattern Diary will be turned in. A physical exam will be performed by the study doctor; the patients may defer the rectal exam if not clinically indicated.An exit interview will be done with study staff.

12 RATIONALE FOR STUDY DESIGN

12.1 Strengths and limitations

Major strengths of this study include the ability to noninvasively measure gastrointestinal transit and the resources available in the Charlton 7 CRU at the Mayo Clinic Rochester. Therefore, completion of this study is highly feasible. The study team has a well-established track record using these techniques in healthy volunteers in previous studies. The study has sufficient power to detect clinically relevant differences between the treatment groups. Variability in body size and weight may present pitfalls in the analysis of the data.

12.2 Anticipated results and significance

We anticipate decreased colonic transit without significant effects upon stomach and small bowel transit. We expect no serious adverse events, though the efficacy of the medication being relief of constipation may result in diarrhea, gas and bloating. We also expect a minor decrease in LDL cholesterol and a slight increase in C4, also consistent with the pharmacologic actions of the medication.

12.3 Feasibility and timeframe

This study requiring four days of investigation in female patients with functional constipation is feasible. Based on past experience, recruitment of study subjects should be uncomplicated.

12.4 Selection of study population

Thirty-six female patients with diagnosed functional constipation will be recruited from the local community by public advertisement placed within areas of Mayo Clinic or by a targeted mailing of an informational letter. They will be screened by means of the Long BDQ to confirm the diagnosis of functional constipation.

This study will reflect the population of Olmsted County, Minnesota with 90% Caucasian, 5% Asian and 5% other. Previous experience would suggest an age range of patients from 18-65 years old, with a mean age of approximately 30 years. No subpopulations or special classes of subjects are involved in this study.

13 INCLUSION CRITERIA

1. Females aged 18 to 65 years old inclusive

2. An adaption of Rome II criteria for functional constipation will be used. Eligible patients must have functional constipation for the previous three months with symptom onset at least six months prior to diagnosis with less than three complete spontaneous bowel movements per week with two or more of the following:
   a. hard or lumpy stools at least 25 % of the time
   b. straining during a bowel movement at least 25 % of the time
   c. sensation of incomplete evacuation at least 25 % of the time
   d. sensation of anorectal obstruction/blockage at least 25 % of the time

3. A normal rectal exam result on file within the past 2 years or performed at Visit 1 screen by a qualified gastroenterologist to exclude the possibility of an evacuation disorder. Examination must exclude findings suggestive of an evacuation disorder such as high sphincter tone at rest, failure of perineal descent and spasm, tenderness or paradoxical contraction of the puborectalis muscles.

4. Females of child-bearing potential (those who have not experienced a bilateral tubal ligation, hysterectomy or menopause) must use an acceptable method of contraception during the study. Acceptable methods are surgical sterilization, hormonal methods such as oral contraceptives, Norplant and Depo-Provera, double barrier method such as a condom and spermicide, and an IUD. Abstinent females may participate if they agree to use the double barrier method should they become sexually active during the study.

5. Able to provide written informed consent prior to any study procedures being performed

14 EXCLUSION CRITERIA

1. Female patients who are pregnant or breast feeding

2. Structural or metabolic diseases/conditions that affect the gastrointestinal system or functional gastrointestinal disorders other than constipation. The long version BDQ will be used to confirm patients have constipation.

3. Taking any medication that in the opinion of the principal investigator has a potential to alter GI transit. This includes but is not limited to osmotic or stimulant laxatives, magnesium or aluminum-containing antacids, prokinetics, erythromycin, narcotics, anticholinergics, selective norepinephrine reuptake inhibitors (SNRIs), opiates, GABAergic agents and benzodiazepines.

   Note: Selective serotonin reuptake inhibitor (SSRI) antidepressants are permissible at low, stable doses. Analgesics such as Tylenol, ibuprofen, naproxen and aspirin are permissible. All medications shall be reviewed by the principal investigator on a case by case basis.

4. Clinical evidence (including but not limited to a clinically significant abnormal physical exam, ECG or laboratory test result in the past medical record) or current clinically significant abnormal physical exam or laboratory test result that could indicate significant cardiovascular, respiratory, renal, hepatic, gastrointestinal, hematological, neurological, psychiatric or other diseases that interfere with the objectives of the study. If a laboratory test result is abnormal and clinically significant, it may be repeated once at the discretion of the PI. If the laboratory test result remains abnormal and clinically significant, the patient will be discontinued from the study and referred to a primary care physician for further evaluation.

5. Patients who are considered by the PI to be alcoholics not in remission or known substance abusers.

6. Patients who have participated in another clinical study in the past 30 days.

15 GENDER/MINORITY MIX

We justify restricting enrollment to only female patients with functional constipation by the following:

1. The clinical population presenting with functional constipation is greater than 90 percent female.

2. The clinical population potentially receiving A3309 as a treatment for functional constipation is mostly female.

3. This is a non-therapeutic study; therefore, we are not depriving males of a potential therapy for functional constipation.

It is anticipated that the racial/ethnic characteristics of the group will reflect the communities in Olmsted County, Minnesota and are anticipated to be 90% Caucasian, 5% Asian and 5% other minorities. The prevalence of IBS among whites, blacks and Hispanics in the U.S. is very similar.

There are no known ethnic or gender differences in bioavailability of A3309. Hence, we anticipate the results of our study will be generalizable.

16 RESTRICTIONS

Patients must report to Charlton 7 CRU fasting for the previous eight hours for all study visits, Visits 1 to 7.

Rescue medications (Milk of Magnesia, Dulcolax and Fleets/tap water enemas) may be used but only under the direction of the PI. Rescue medications must not be used within the seven days prior to both the abbreviated and full transit scintigraphy tests. Since each patient's schedule will differ, each patient will be instructed as to when rescue medication may not be taken.

17 STUDY WITHDRAWAL

A study patient should be withdrawn from the study treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the expressed wish of the patient.

Irrespective of the reason for withdrawal and whenever possible, the patient should be examined. Relevant laboratory test samples should be obtained and all relevant assessments should be completed, preferably according to the scheme for the final assessment. The CRF should be completed as far as possible and collected by the Monitor.

A withdrawn patient is not allowed to re-enter into the study.

18 SCREENING AND ENROLLMENT LOG

The site will keep a log of all patients screened and included. The reason for screen failure should be stated for all patients screened but not included. The reason for withdrawal should be stated for all patients included but not completed.

19 INVESTIGATIONAL PRODUCT

Investigational Product (IP) will be provided by Albireo. The study will be performed under an IND held by Albireo, IND number 103,060.

The active pharmaceutical ingredient (API) in A3309 is manufactured by Syngene as a white to off-white powder. The IP will be shipped from Galenica to the Mayo Research Pharmacy.

19.1 Packaging, labeling and storage of Investigational Product

The A3309 tablets will be delivered to the Mayo Research Pharmacy in aluminium sachets. The sachets are packed in HDPE containers (DUMA Twist-Off) with childproof polypropylene caps (DUMA Twist-Off cap).

The label of the sachets (primary container) will include:

Content: *A3309 tablets and/ or Placebo tablets*
Protocol Number: *A3309-003*

Storage: *Room Temperature (preferably 20 – 25˚C)*
Batch No: *96202-YYMM-XX*
Use by: *YYYY-MM-DD*
Caution: New drug - Limited by Federal (USA) Law to Investigational Use
Sponsor: *Albireo AB, Arvid Wallgrens backe 20, SE-413 46 Gothenburg, Sweden*
Keep Out of Reach of Children.
Take both tablets orally daily before breakfast

The label of the HDPE containers (secondary container) will include:

18 sachets containing two tablets each.
Content: *A3309 tablets and/or Placebo tablets*
Protocol Number: *A3309-003*
Patient no: *XXXX*
Storage: *Room Temperature (preferably 20 – 25˚C)*
Batch No: *96202-YYMM-XX*
Use by: *YYYY-MM-DD*
Caution: New drug - Limited by Federal (USA) Law to Investigational Use
Sponsor: *Albireo AB, Arvid Wallgrens backe 20, SE-413 46 Gothenburg, Sweden*
Manufactured by: *Galenica AB, Medeon, SE-205 12 Malmoe, Sweden*
Clinical site*: Mayo Clinics, xxxxxxxxxx, phone: xxxxxx*
Keep Out of Reach of Children.
Take both tablets from one sachet orally daily before breakfast.

19.2 Doses and treatment regimens

Patients will receive placebo or 15 mg or 20 mg A3309 tablets taken orally once daily for eleven consecutive days at home and once daily for three consecutive days of the final transit scintigraphy test at the Charlton 7 CRU. Study medication will be administered at Charlton 7 CRU by CRU nurses.

Based on the effect on the biomarker C4, data on colon transit, and results of patient-reported outcomes in study A3309-001, the protocol design includes two dose levels: 15 and 20 mg.

These dose estimations are based on the following findings from preclinical studies:

- PD effects of A3309 tested in several species in both *in vitro* and *in vivo* experiments. The *in vitro* potency in human IBAT-transfected cells, as well as the *in vitro* and *in vivo* potency in the mouse and the dog were used to approximate an effective dose in humans from 0.25 to 0.5 μmol/kg.
- A dose of A3309 0.16 μmol/kg resulted in twice the fecal excretion of ^{75}SeHCAT in the mouse than in humans over a 24-hour period; mouse IBAT showed a 3-fold greater potency than human IBAT. In the dog, a clear increase of C4 and in diarrhea was observed and a reversal of meat-induced constipation was detected at 5 μmol/kg (the canine IBAT has a 10-fold less potency than human IBAT). As diarrhea was observed in the dog at 5 μmol/kg in the 3 month toxicology study, this dose is probably too high for use in patients with constipation.
- Evaluation of the results of the A3309-001 study showed:

- A minor signal on C4 and on lipid parameters observed at the 3 mg and the 10 mg dose levels produced a stronger signal;
- Efficacy signals (colon transit, stool form scale (28), and frequency of BMs) evident in the 10 mg dose group. However, no diarrhea or incapacitating "very loose stools" were observed in the 10 mg dose group.

Given the paucity of data, the therapeutic oral dose range in man is difficult to estimate but may be in the range of 5 to 20 mg, as calculated from *in vitro* potency in human IBAT-transfected cells and from *in vitro* and *in vivo* potency in the mouse, dog, and man.

Previous experience with A3309 does not include dose levels greater than 10 mg. Evaluating 15 mg and a 20 mg dose level is adequate given:

- The large safety window in the preclinical toxicology package. The only dose-limiting effect identified was emesis in female dogs given high dose. The preliminary NOAEL in the rat was 350 mg/kg, 140 mg/kg in the male dog, and 17.5 mg/kg in the female dog, giving an approximate safety margins of approximately 520 to 1300-fold differences for a 20 mg dose (see Section 1.2.6).
- The extremely low systemic exposure (picomolar exposure) of both A3309 and its metabolites
- The high protein binding (approximately 99.5 %), and
- The benign safety profile in the Phase Ib study

The available toxicological documentation indicates that A3309 can be given orally to humans for up to 3 months duration at doses of 15 mg and potentially within the range of 1 to 20 mg for up to 90 days. In parallel with this study a Phase IIb study (A3309-002) is ongoing in the US including three dose levels (5, 10 and 15 mg) and with a treatment duration of 8 weeks. Based on the results of that study and the results of this study data will be evaluated and a decision taken whether to continue into a Phase 3 program.

19.3 Product accountability

The IP will be released to the study site after approvals of the study protocol have been received from the IRB and the Regulatory Authority. The IP will be stored at and dispensed by the Mayo Research Pharmacy and administered by the Charlton 7 CRU nurses.

The Mayo Research Pharmacy will be responsible for keeping detailed dispensation records, which show the quantity of IP that is stored, removed from the place of storage, prepared for delivery to Charlton 7 CRU and any unused product returned from Charlton 7 CRU. Any discrepancies between dispensed and returned IP must be explained and documented.

The CRU will keep record of IP administered to the patients.

IPs deliberately and/or accidentally destroyed by the Research Pharmacy or the dispensing nurses must also be accounted for.

The Monitor will perform IP accountability and make sure that all unused IP is adequately destroyed/returned and documented.

20 RANDOMIZATION METHODS

Randomization will be stratified on BMI (< 25, > = 25 kg/m^2). IP doses will be randomly assigned in fixed block sizes according to a schedule provided by the study statistician. Mayo Clinic Research Pharmacy will maintain the randomization schedule.

20.1 Blinding

The different doses of IP and placebo will be of identical appearance. Only pharmacy personnel will be allowed to see the randomization schedule and know the block size to avoid the possibility of predication by the Investigators and study staff. All study personnel (including the study statistician) will remain blinded to the assignments until the study is completed and the analysis data set is finalized.

20.2 Emergency decoding of blinded treatment

Breaking the code must only be done in emergency situations and should only be used when knowledge of the treatment is necessary for the proper management of the patient. If the treatment code is broken, the reason and the date must be documented. Decoding of the blinded treatment must be reported to the Albireo AB, the CRO Icon and Mayo IRB.

20.3 Prior and concomitant therapy

Rescue medications and other medications considered necessary for the patient's welfare may be given at the discretion of the Investigator.

All concomitant therapy used during and within 30 days of study entry must be recorded in the CRF. No other drug under investigation may be used concomitantly with the study medication.

20.4 Continuation of treatment

Continuation of treatment with the IP after the study period will not be offered.

20.5 Treatment Compliance

Treatment compliance of greater than 80% is required and will be assessed by the patient's record of dosing contained in the Bowel Pattern Diary.

21 STUDY ASSESSMENTS

21.1 Demographics

At Visit 1 the following information will be collected and registered in the CRF: age, height, weight, BMI, gender and ethnic origin.

21.2 Clinical efficacy assessments

1. Gastrointestinal and Colonic Transit

An adaptation of our established scintigraphic method will be used to measure gastrointestinal and colonic transit. Briefly, ^{111}In absorbed on activated charcoal particles is delivered to the colon by means of a methacrylate-coated, delayed-release capsule. The capsule is ingested following an overnight fast. Colonic transit measurements will be performed by means of the delayed release capsule. After the capsule emptied from the stomach (documented by its position relative to radioisotopic markers placed on the anterior iliac crests), the study medication is given immediately before a standard radiolabeled breakfast meal is ingested. In this meal, ^{99m}Tc-sulfur colloid is used to label two scrambled eggs that will be eaten with one slice of whole wheat bread and one glass of skim milk. This meal facilitates measurement of gastric and small bowel transit.

Relative to the time of consumption of breakfast meal, abdominal camera images are initially obtained every 30 minutes for the first two hours, then hourly for the next six hours. At about 4 hours, a standard chicken breast lunch will be given. Camera images will be taken at 5, 6, and at 8 hours post breakfast meal. At about 8 hours, the standard roast beef sandwich snack will be given. The patient will leave the CRU after the completion of the 8 hour image and will return the following two mornings in order to receive study medication and obtain 24 and 48 hour scans fifteen minutes later. The performance characteristics of this test are summarized elsewhere. Please see relevant references on the methods (29,30).

2. Assessment of stool frequency and consistency

During the study, patients will complete a daily Bowel Pattern Diary to record their bowel habits. The Bowel Pattern Diary will be dispensed at Visit 1 and will be collected at the completion of the study at Visit 7.

3. Pharmacokinetic (PK) and pharmacodynamic (PD) assessments

a. Sample collection and handling

Blood samples for analysis of PK parameters will be collected at Visit 4, before dosing and at 30, 60, 90, 120, 180, 240, 360 and 480 minutes post-dose. One solitary sample to measure a baseline level at Visit 1 and one sample to measure a post treatment level at Visit 7 will be obtained. The samples will be drawn from an IV line into a lavender EDTA collection tube, centrifuged at 3000 rpm for 15 minutes and the plasma transferred to an anonymized polypropylene tube labeled only with a study number and patient initials as identifiers. The samples will be frozen at -70 degrees Celsius until they are batch shipped to Covance UK for analysis after the end of the study. One 5-mL pharmacodynamic sample to determine levels of C4 is taken at Visit 1, 4 and 7 and stored in the refrigerator for 30-60 min and thereafter serum is separated by centrifugation. The serum obtained is aliquoted into a cryo tube equipped with a screw-cap lid with a rubber o-ring. Samples are thereafter frozen at -70 degrees Celsius until they are batch shipped to Karolinska Institue, Sweden for analysis after end of the study. The total amount of blood drawn for these pharmacokinetic and pharmacodynamic samples is about 75 mL.

b. Pharmacokinetic analysis

PK parameters will be analyzed using conventional analyses by Covance, UK:

21.3 Laboratory safety assessments

Blood and urine samples will be obtained for the standard laboratory tests safety assessments of CBC, CMP, coagulation studies and UA at Visit 1, Visit 6 and Visit 7. A urine screen for drugs of abuse will be performed at Visit 1. These laboratory panels are further described in Appendix 3. The total amount of blood drawn will be approximately 20 mL on each visit for a total of 60 mL. Urine pregnancy tests will be performed for females of child bearing potential at Visit 1 and within 48 hours prior to the receipt of

radiation for both the abbreviated and full transit scintigraphy tests performed at Visit 2 and Visit 4, respectively. The laboratory analysis will be performed at Mayo Clinic.

21.4 Clinical safety assessments

A physical examination will be performed at the Visit 1 study entry and Visit 7 study completion and if necessary throughout the study.

Vital signs (including temperature, pulse, blood pressure and respiration rate) will be recorded at all visits.

Patients will be interviewed about adverse events and concomitant medications at every visit.

22 ADVERSE EVENTS

22.1 Definitions

Adverse Events (AE)

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

Serious Adverse Event (SAE)

Any untoward medical occurrence or effect that at any dose

- results in death
- is life-threatening
- requires patient's hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability or incapacity
- is a congenital anomaly/birth defect
- is regarded as medically important without meeting the above mentioned criteria.

The term life-threatening in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

Hospitalization is defined as an unplanned overnight stay. Note however that the subject must be formally admitted. Waiting in outpatient clinic or emergency room would not count. A planned hospital stay does not count as SAE, nor does staying in hospital for social reasons (e.g. respite care, the fact that there is no-one at home to care for the patient).

Unexpected Adverse Drug experience

Any adverse drug experience, the specificity or severity of which is not consistent with the current Investigator Brochure (IB); or, if an IB is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Intensity

Mild means awareness of symptoms or signs, but easily tolerated (acceptable)

Moderate means discomfort enough to interfere with usual activity (disturbing)

Severe means incapacity to work or to do usual activity (unacceptable)

Causality

Certain - A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary.

Probable - A clinical event - including laboratory test abnormality - with a reasonable time sequence to use of the product, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required.

Possible - A clinical event - including laboratory test abnormality - with a reasonable time sequence to use of the product, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear.

Unlikely - A clinical event - including laboratory test abnormality - with a temporal relationship with use of the product which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations.

Not related - Evidence exists that the AE has an etiology other than the study drug (e.g., pre-existing condition, underlying disease, intercurrent illness, or concomitant medication).

An adverse event will be considered associated with the use of a study drug if the attribution is certain, probable or possible.

22.2 Evaluations

All AEs, serious and non-serious, should be recorded in the CRF. If no AE has occurred during the study period, this should also be recorded in the CRF.

The following evaluations are to be done by the Investigator in connection with the AE:

- type of event
- seriousness
- degree of severity
- duration (start - end)
- action taken
- causality with study product
- outcome of the adverse event

For AE reporting purposes no distinction should be made between the IP and any reference/comparator product.

22.3 Reporting of SAEs

All SAEs must be reported by the Investigator using phone or fax within 24 hours of knowledge of the event to the Monitor or other members of the staff at the CRO Icon, regardless of the time that may have elapsed from the time the event occurred to when the Investigator first learns of it. The Initial Report should contain as a minimum the following information:

- subject identification
- treatment specification
- adverse event diagnosis
- time specification for the medical event
- name of the original reporter

A Serious Adverse Event Report Form must also be completed, signed by the Investigator and submitted to the CRO Icon no later than five calendar days after the initial information was received. Apart from the information above, this Follow-up Report should also contain the following information:

- assessment of severity
- assessment of causality

No distinction should be made between the IP and the reference product regarding reporting of SAEs as long as the code is not broken.

SAEs should be reported to the CRO Icon even after the clinical study has been finished, if, in the judgment of the Investigator, there might be an association between the event and the previous use of the study product(s) or as a result of the study procedures. This post-study observation period will be up to 30 days after administration of last dose of study product.

An SAE may qualify for reporting to the FDA by the Sponsor or Sponsor's representative if the AE is both serious and unexpected based upon the current Investigator's Brochure. In this case, all Investigators will receive a formal notification describing the SAE in the form of an IND safety report.

22.4 Exceptions from expedited reporting of SAEs

An event need not to be reported as an SAE if it represents only a relapse or an expected change, or progression of a pre-existing condition and is without any other symptoms or signs than those present before treatment. This event needs only to be reported as an AE and should be described in the Study Protocol.

22.5 Follow-up period after an AE

If a study patient is withdrawn due to an AE, or if an AE persists at the end of the study treatment period, this should be followed up by a physician until the condition has ceased or until the subject is under professional medical care and a potential causality between the investigational drug and the AE has been penetrated. An outcome assessment should be performed when an AE persists.

22.6 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the study treatment must be stopped immediately, and the patient discontinued from participation in the study. Pregnancy itself is not regarded

as an AE unless there is a suspicion that the IP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the patient was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as AEs. All outcomes of pregnancy must be reported to the CRO Icon on the pregnancy outcomes report form.

22.7 Procedures in case of overdose

Overdose is defined as any dose greater than the highest daily dose in the protocol-defined doses, ie, 20 mg. Any overdose must be recorded in the study medication section of the CRF. At least 12 hours should elapse between consecutive calendar days and no more than one dose should be taken per calendar day. In case of overdose, appropriate treatment should be given and hospitalization for observation should be considered. Though an overdose may not be considered an SAE, any case of reported overdose must be reported to the sponsor and CRO in the same expedited manner as an SAE using an SAE form. If the PI and/or sponsor and CRO consider the overdose to be medically significant, it would then be reported as an SAE.

23 DATA QUALITY ASSURANCE

23.1 Monitoring and auditing procedures

The study site will be visited by the Monitor and/or the Project Manager periodically at times agreed on by the Investigator and the Monitor. It is the function of the Monitor to ascertain that all aspects of the Study Protocol are complied with and that the conduct of the study conforms to applicable regulatory requirements and established rules for Good Clinical Practice (GCP).

At the time of each monitoring visit, the Monitor will review the completed CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the protocol.

The Monitor will also check that the data in the CRF are consistent with the clinical records (Source Data Verification) and that study results are recorded completely and correctly. The Monitor will check on the reporting of SAEs and the procedures for IP accountability and record keeping. For this purpose the Monitor must be given direct access to clinical records, original laboratory data, etc., as far as these relate to the study and without jeopardizing subject integrity. CRFs for all included subjects must be made available to the Monitor for review.

The study site may also be subject to quality assurance audit by the Sponsor or someone appointed for this task by the Sponsor. A Regulatory Authority may request to make an inspection of the study site. The procedures of such a visit would be similar to those of a monitoring visit, and data already checked by the Monitor may be checked again. The Investigator is required to inform the Sponsor immediately of an inspection requested by a Regulatory Authority.

23.2 Case Report Forms (CRFs)

CRFs of a design mutually agreed upon by the Sponsor, the CRO and the PI will be developed by the Mayo Clinic.

A CRF must be completed and signed for each included subject. All data and information on the CRFs are to be neatly and legibly recorded in black or blue ball-point ink to ensure legibility of self-copying and photocopied pages. Corrections on the CRFs must be made by one single line through the incorrect data, leaving the corrected data clearly visible. The revised entry should be made alongside, initialed and dated by a member of the PI's research team who is authorized to initial CRF changes for the Investigator. This authorization must be documented in writing. Correction fluids are not permitted.

The completed CRFs should be made available for checking of completeness and accuracy before collection by the Monitor as agreed in advance. The original CRFs are the sole property of the Sponsor and should not be copied or made available in any form to a third party, with the exception of authorized representatives of local Regulatory Authorities, without the written permission from the Sponsor.

23.3 Source Data

Source documents will be the Mayo Clinic EMR, original laboratory results and original electronic data generated from the scintigraphic investigations. Relevant EMR for study patients will be printed in certified hardcopies prior to the monitoring visit and used for SDV.

The extent of SDV to be performed will be described in the study specific monitoring instructions prior to initiation of the study.

The source of derived information appearing in the CRF will be included in each individual CRF binder for monitoring purposes. The SDV process will be performed continuously during the monitoring visits at the clinic.

23.4 Training of study staff

It is the responsibility of the PI to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, and have a detailed knowledge of and training in the procedures that are to be executed by them.

All Investigators and staff carrying out observations of primary or other major efficacy variables involved in the study should provide a Curriculum Vitae (CV). The PI will keep a list of all personnel involved in the study together with their function and study related duties delegated. He/she will ensure that appropriate study related training is given to all of these staff, and that any new information of relevance to the performance of this study is forwarded to the staff involved.

Before inclusion of subjects the Monitor and/or Project Manager will perform a study initiation visit to inform and train relevant study staff.

24 STATISTICAL METHODS

24.1 Statistical considerations

An analysis of covariance (ANCOVA) will be used to compare transit parameters and patient bowel pattern diary summaries (i.e., stool frequency, mean stool consistency and mean ease of passage scores) among the treatment groups. The covariates considered for inclusion in the analyses are age, body mass index (BMI) and the corresponding baseline (pre-treatment) bowel pattern diary summaries. If necessary a suitable transformation for potential skewness in the distributions of measured responses or heterogenous variation amongst the treatment groups may be used (i.e., ANCOVA on ranks or square root transformed stool frequency.

If the ANCOVA test for overall treatment effects for a given response has a p value less than or equal to 0.10, then both the 15 mg and 20 mg doses will be compared to placebo using Dunnett's Test.

Since each of the primary endpoints assesses a separate hypothesis regarding the effects of A3309, no adjustment in the alpha level for testing multiple types of endpoints is anticipated, and a two-sided significance level of 0.05 will be used in each ANCOVA model.

24.2 Analysis data sets

The primary analyses will follow the intent to treat (ITT) paradigm with all patients randomized included in the analyses. Those patients with missing response values will have their missing values imputed via the overall (patients with non-missing data) mean and a corresponding adjustment in the ANCOVA residual error variance degrees of freedom (subtracting one for each missing value imputed). A per protocol analysis of transit data will also be performed. The per protocol population consists of all patients having at least 80 % compliance with study drug and having participated in the transit evaluation.

Safety data will be presented for all patients receiving investigational product.

24.3 Demographics

The following baseline values and patient characteristics will be summarized descriptively in order to describe the study population: age, gender and ethnic origin.

24.4 Analysis of efficacy

Primary endpoint:
Colonic geometric center at 24 h as measured by scintigraphy, as compared to placebo.

Secondary endpoints:
1. Colonic filling at 6 h measured by scintigraphy, as compared to placebo.
2. $T_{1/2}$ of ascending colon emptying measured by scintigraphy, as compared to placebo.
3. Colonic geometric center at 48 h measured by scintigraphy, as compared to placebo.
4. $T_{1/2}$ of gastric emptying of solids
5. Assess the effects on stool frequency and consistency based on the Bowel Pattern Diary and treatment effects on symptom responses specific to constipation, overall and for active treatment compared to placebo.
6. Evaluate the pharmacokinetic (PK) characteristics for each dose of A3309.
7. Assess the safety and tolerability of A3309 administered as an oral tablet.

24.5 Transit data analysis

A variable region of interest program is used to quantitate the counts in the stomach and each of four colonic regions: ascending, transverse, descending, and combined sigmoid and rectum. These counts were corrected for isotope decay, tissue attenuation, and downscatter of ^{111} In counts in the ^{99m}Tc window.

Data will be analyzed as in previous studies.

1. Geometric mean of counts in anterior and posterior gastric regions of interest will be used to estimate by power exponential analysis of the proportionate emptying over time of counts from ^{99m}Tc solids and ^{111}In from the stomach. The proportion of ^{99m}Tc and ^{111}In reaching

the colon at 6 hours will also be estimated as a measure of orocecal (and a surrogate for small bowel) transit.

2. Geometric center of colonic counts for solids at 4, 24 and 48 hours will be estimated using geometric mean of counts in ascending, transverse, descending and rectosigmoid colon and stool (weighted by factors of 1 to 5 respectively). The primary variable of interest in overall colonic transit is the geometric center at 24 hours.

3. Ascending colon emptying t1/2 will be estimated by power exponential analysis of the proportionate emptying over time of counts from the colon. The primary data for this analysis will be the proportion of decay and depth-corrected counts in the ascending colon on the hourly scans on the first day of transit measurement and the 24 h data.

The primary summaries obtained for comparison of transit profiles will be the colonic geometric center at 24 hours and the ascending colon emptying t½. The geometric center is the weighted average of counts in the different colonic regions: ascending (AC), transverse (TC), descending (DC), rectosigmoid (RS), and stool. At any time, the portion of colonic counts in each colonic region is multiplied by its weighting factors as follows: (%AC x 1 + %TC x 2 + %DC x 3 + %RS x 4 + %stool x 5)/100 = geometric center. Thus, a high geometric center implies faster colonic transit. A geometric center of 1 implies that all isotope is in the ascending colon and a geometric center of 5 implies that all isotope is in the stool. The t½ of ascending colon emptying is also estimated by plotting the activity-time curve for percent residing in the ascending colon; linear interpolation is used to connect points.

24.6 Stool frequency and stool consistency

Patients are asked to keep a daily bowel pattern diary documenting the number of stools and the consistency (Bristol Stool Scale (28)) of each stool. These are averaged as number of bowel movements or consistency of bowel movements per day per patient. Number of bowel movements and consistency are subsequently averaged over the study periods (i.e. run-in and treatment) per patient. The ANCOVA will be used to assess treatment effects on each of these two measures separately, using the corresponding run-in values as covariates. In addition, treatment effects on specific constipation symptoms , considered as discrete categorical responses, will be evaluated by a contingency table analysis of the post-treatment responses based on the final post-treatment bowel pattern diaries and questionnaires concerning constipation symptoms and treatment efficacy.

24.7 Analysis of pharmacokinetic and pharmacodynamic data

Pharmacokinetic and pharmacodynamic parameters will be analyzed as described in the SAP.

24.8 Analysis of safety

All safety variables and AEs will be presented, when possible, by treatment, with summary statistics.

All continuous variables will be described using standard statistical measures, i.e. number of observations, number of missing observations, mean, standard deviation, minimum and maximum value, median, 1st and 3rd quartile. All categorical variables will be summarized by absolute and relative frequencies.

24.9 Assessment of sample size

Table 1 below summarizes data for the primary response measures and uses the (relative) variation, (CV%) to estimate the effect size detectable with 80% power based on a two sample t-test at a two-sided alpha level of 0.05. The effect size is the difference in group means as a percentage of the overall mean for each response and assumes 12 subjects per group. The ANCOVA should provide 80% power to detect similar (pairwise) differences using a pooled estimate of variation across all three groups and potentially even smaller effect sizes by adjusting for important covariates. The data from the scintigraphic transit studies are based on the same methods proposed for this study. There is no planned adjustment for dropouts. In these types of studies, we expect less than five percent dropouts. Missing data will be handled by standard imputation methods (e.g. use of mean data for all participants for that specific missing data), with appropriate adjustment of the degrees of freedom for each data imputed.

Table 6 Primary Response Measurement Summary

Assuming n=12/ group		**Effect Size‡ (%) Detectable(N=12 per group) with:**
Response Type	**CV%**	**80%Power (α=0.05)**
GE solids $t_{1/2}$ min†	25	27
GC @ 24 hrs †	35	37

‡ Effect Size is the difference between means as a percentage of listed mean

† * Based on data from previous studies (31,32,33) using similar methods to those proposed here. Transit data† from C-IBS studies (e.g. Renzapride, prucalopride, Tegaserod).

24.10 Data Management

Standardized CRFs and patient diaries will be developed in accordance with the study protocol. The PI will ensure that all data from patient visits including details of AEs and concomitant medications are entered promptly in the CRFs in accordance with specific instructions given. The PI must sign each CRF to verify the integrity of the data recorded. All scintigraphy data, laboratory values and patient diary data will be provided to the Sponsor electronically in the form of Excel spreadsheets.

The PI, Statistician and nuclear medicine technician will quality check the scintigraphy data before database lock. Audits of data may be conducted at the discretion of the sponsor Albireo AB by its representatives and/or regulatory agencies such as the FDA and Mayo IRB.

The database will be locked when clean file has been declared. Any changes to the database after that time can only be made by joint written agreement between the Sponsor, the Statistician and the PI.

The computerized data processing and the statistical analysis will be performed by the Mayo Clinic. Data will be processed in agreement with local legislations.

24.11 Changes in the approved study protocol

Any proposed change to the study protocol version reviewed and approved by FDA and IRB (including appendices) will be documented in a written and numbered protocol amendment. All amendments must be submitted to Mayo IRB and Regulatory Authority for approval, according to applicable national regulations. A substantial protocol amendment should be signed and dated by the same parties who signed the Final Study Protocol, as applicable.

25 EMERGENCY PROCEDURES

25.1 Emergency contacts

In case of an emergency the Investigator should contact the Monitor at the CRO Icon or the Project Manager at Albireo.

25.2 Procedures in case of medical emergency

The PI is responsible for ensuring that there are procedures and expertise available to cope with medical emergencies during the study.

26 STUDY TIME TABLE

The first patient will be screened during January 2010 and the last patient will be completed during November/December 2010. The end of the clinical phase of the study will be defined as the last patient's last study visit. The final closure of the study will be declared when Clinical Study Report has been finalized at Albireo AB and Mayo IRB has reviewed and approved a Final Progress Report which will not occur until a manuscript has been accepted for publication.

27 DISCONTINUATION OF THE STUDY

The Sponsor reserves the right to discontinue the study at any time, but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must call in all participating subjects within one month and inform the subjects as well as perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused IPs and other study materials must be returned and all CRFs completed as far as possible.

Investigator(s) will be reimbursed for reasonable expenses incurred, in the event this becomes necessary.

28 FINAL STUDY REPORT AND PUBLICATION OF STUDY RESULTS

After conclusion of the study, Mayo will analyze the data and write a manuscript for publication in a peer-reviewed scientific journal. The Sponsor will have the opportunity to review the study data according to the conditions stipulated in the contract developed by Mayo's legal section. Publication rights are specifically identified in the Mayo Legal Section contracts.

A Clinical Study Report, in compliance with ICH E3; *Structure and content of Clinical Study Reports*, describing the conduct of the study, the statistical analysis performed and the results obtained will be prepared by the Sponsor or designee.

A summarizing report will be submitted to Regulatory Authorities and IRB within 12 months from end of study. The Final study Report will be submitted to Regulatory Authorities when available.

29 RECORD RETENTION

The Investigator must arrange for retention at the investigational site of a list of the subjects and their identifying code, subject files and other study documents. The archiving period must be adapted to

regulations in force and should not be shorter than ten years after the termination of the study and the presentation of the final report.

It is the responsibility of the Sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

30 DISCLOSURE AND CONFIDENTIALITY

All unpublished information concerning the test product and research carried out by the Sponsor, including patent applications, manufacturing processes, basic scientific data, etc., is considered confidential and the sole property of the Sponsor. Disclosure to third parties must be limited to those undertaking legitimate peer review of the scientific and ethical aspects of the study and to those participating, including the recipients of drugs, so that customary medical care and informed consent can be achieved.

31 INSURANCE/INDEMNITY

As described in the Research Agreement between Albireo AB and the Mayo Clinic, each party agrees to maintain, at its own expense, insurance in quantities and types, or self-insurance, sufficient to meet its legal obligations hereunder. All policies shall be issued by financial secure companies and shall be written as primary coverage. Such insurance shall provide for 30 days' prior written notice of cancellation or material change. Upon request, each party shall provide the other party with certificates of insurance evidence coverage.

32 STUDY AGREEMENTS

The Principal Investigator at the investigational site must comply with all the terms, conditions, and obligations of the Research Agreement for this study. In the event of any inconsistency between the Study Protocol and the Research Agreement, the Research Agreement shall prevail. Financial terms and conditions are specified in an exhibit to the Research Agreement. A separate financial agreement between Albireo AB and Mayo Clinic will be filed with Research Administration and a legal contract with the Mayo Legal Department as per standard protocol. The Principal Investigator is required to maintain an updated Regulatory Binder which includes all required documents as per the Mayo Office of Research Compliance.

33 REFERENCES

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34 APPENDICES

34.1 Study Flow Chart

Study assessments are described in the chart below.

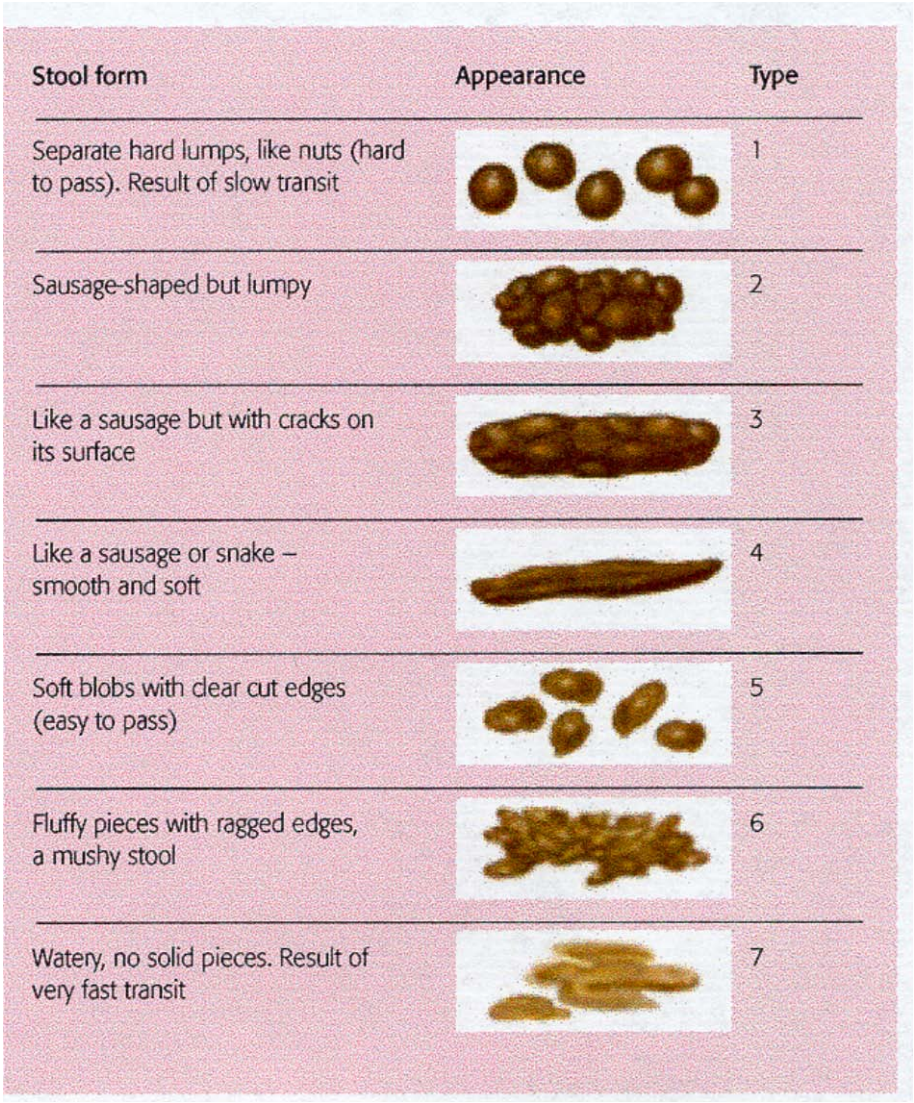
	V1	V2	V3	V4-V6	V7
Event	**Screen**	**Baseline Transit (V1 + 7-14 days)**	**Randomization (V2 + 0-3 days)**	**Final Transit (days 12-14 of study med)**	**Follow-up (V6 + 7-10 days)**

Patient information	x				
Informed consent	x				
Inclusion/exclusion criteria	x				
Demographics	x				
Medical history	x				
Physical examination[1]	x			x	x
Height/Weight	x				x
Vital signs[2]	x	x	x	x	x
Med history/adverse events[3]	x	x	x	x	x
Concomitant medication	x	x	x	x	x
Laboratory safety tests[4]	x			x	x
Pharmacodynamics[5]	x			x	x
Pregnancy test[6]	x	x		x	
12-lead ECG	x			x	x
Long BDQ	x				
Bowel pattern diary	x (out)				x (in)
Symptom and efficacy survey[7]			x	x	
Scintigraphy[8]		x		x	
Randomization[9]			x		
Pharmacokinetics[10]	x			x	x
Administration of study med			x	x	

[1] A rectal exam to be performed at Visit 1 to exclude the possibility of an evacuation disorder if a normal exam not documented within 2 years by a qualified gastroenterologist A patient may defer the rectal exam at Visit 6 and 7 if not clinically indicated.
[2] Includes temperature, blood pressure, pulse and respiratory rate
[3] Adverse events occurring prior to administration of study medication are classified as medical history
[4] Laboratory safety assessments include clinical chemistry (ALT; Albumin; Alkaline phosphatase; AST; Bilirubin, total; Calcium; Chloride; C-Reactive Protein; Creatinine; GGT; Glucose; Potassium; Protein, total; Sodium; Urea; Uric acid), hematology (hemoglobin; hematocrit; MCV; platelet count; RBC count; WBC count with differential), coagulation studies (Activated partial prothrombin time aPPT; Prothrombin time PT), urinalysis (blood; glucose; ketones; leukocytes; nitrites; pH; protein), drugs of abuse urine screen (alcohol; amphetamines; barbiturates; benzodiazepines; cocaine; opiates; phencyclidine; tetrohydrocannibinol). The drugs of abuse urine screen is performed once at Visit 1; the other laboratory tests are performed at Visit 1, Visit 6 and Visit 7.
[5] Blood samples for pharmacodynamics including C4 and a lipid panel of total cholesterol, HDL, LDL and triglycerides are obtained at Visits 1, 4 and 7.
[6]Females of child bearing potential are required to have a negative pregnancy test result within 48 hours prior to the receipt of radiation. Females who are status post-tubal ligation, post-hysterectomy or post-menopause are exempted from this requirement
[7] A six question survey concerning constipation symptoms and treatment efficacy will be given at Visit 3, the end of the pre-treatment period, and again at Visit 6, the end of the treatment period.

[8]An abbreviated transit scintigraphy with scans obtained at 4 and 24h is performed at Visit 2. A full transit with scans obtained at 0, 30 min, 60 min, 90 min, 2, 3, 4, 5, 6, 7 and 8 hours after taking study medication for 12 consecutive days at Visits 4-6.
[9]Criteria for randomization is defined as a geometric center < 2.30 at 24h, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies)
[10]Blood samples for pharmacokinetics are obtained at Visit 4 at pre-dose, 30, 60, 90, 120, 180, 240, 360, 480 minutes after study medication. One blood sample for pharmacokinetics is obtained at Visit 1 and Visit 7 as well.

34.2 Bristol Stool Scale



Ease of Passage:

1. Manual disimpaction required
2. Enema or suppository required to initiate bowel movement
3. Some straining necessary to pass bowel movement
4. Easy normal passage of stool without straining
5. Urgent need to pass bowel movement spontaneously; **no** abdominal pain or discomfort present

6. Urgent need to pass bowel movement spontaneously; abdominal pain or cramping present
7. Incontinent of bowel movements

34.3 Laboratory Test Panels

Complete Blood Count (CBC)

Test	Normal ranges	Units
Hemoglobin	12.0 - 15.5	g/dL
Hematocrit	34.9 - 44.5	%
Erythrocytes	3.90 - 5.03	x 10^{12}/L
MCV	81.6 - 98.3	fL
RBC Distrb Wid	11.9 - 15.5	%
Leukocytes	3.5 -10.5	x 10^{9}/L
Platelet Count	150 - 450	x 10^{9}/L
Neutrophils	1.7 - 7.0	x 10^{9}/L
Lymphocytes	0.9 - 2.9	x 10^{9}/L
Monocytes	0.3 - 0.9	x 10^{9}/L
Eosinophils	0.05 - 0.50	x 10^{9}/L
Basophils	0 - 0.3	x 10^{9}/L

Comprehensive Metabolic Panel (CMP)

Test		Normal ranges	Units
Sodium		135 - 145	mmol/L
Potassium		3.6 - 5.2	mmol/L
Calcium		8.9 - 10.1	mg/dL
Protein,Total		6.3 - 7.9	g/dL
Glucose		70 - 100	mg/dL
Alk Phosphatase	Age	Age dependent	U/L
	16	61 - 264	
	23	52 - 144	
	45	37 - 98	
	50	39 - 100	
	55	41 - 108	
	60	46 - 118	
	65	50 - 130	
AST (GOT)		8 - 43	U/L
ALT (GPT)		7 - 45	U/L
Bilirubin,Total		0.1 - 1.0	mg/dL
Creatinine		0.6 - 1.1	mg/dL
Albumin		3.5 - 5.0	g/dL
BUN		6 - 21	mg/dL
Chloride		100 - 108	mmol/L
Bicarbonate		22 - 29	mmol/L

Other Blood Values

Test	Normal ranges	Units
C-reactive protein	< = 8.0	mg/L
GGT	6 -29	U/L
Uric acid	2.7 – 6.1	mg/dL
aPTT	21 - 33	sec
PT	8.3 – 10.8	sec
Cholesterol	< 230	mg/dL
HDL cholesterol	> 40	mg/dL
LDL cholesterol	< 159	mg/dL
Triglycerides	< 199	mg/dL

Dipstick Urinalysis (UA)

Test	Normal ranges	Units
pH	5.0 – 8.0	pH units
Glucose	Neg	
Protein	Neg	
Hemoglobin	Neg	
Ketones	Neg	
Leukocytes	Neg	
Nitrites	Neg	

Drugs of Abuse urine screen (DAUS)

Test	Normal ranges	Units
Alcohol	< 30	mg/dL
Amphetamine	< 1000	ng/mL
Barbiturates	< 200	ng/mL
Benzodiazepines	< 200	ng/dL
Cocaine	< 300	ng/mL
Opiates	< 300	ng/mL
Phencyclidine	< 25	ng/mL
Tetrahydrocannabinol	< 20	ng/mL

34.4 Patient informed consent form

Name and Clinic Number

IRB # _____
Consent form approved _____;
This consent valid through _____;

1. General Information About This Research Study

Study Title: A Phase II, Double Blind, Randomized, Placebo Controlled Study of the Effects of A 3309, an Ileal Bile Acid Transport Inhibitor, on Small Bowel and Colonic Motor Functions in Female Patients with Functional Constipation.

Name of Principal Investigator on this Study: Dr Michael Camilleri and Colleagues

A. Study Eligibility and Purpose

You are being asked to take part in this research study because we are studying the effects of an investigational (not FDA approved) medication, A3309, on the movement of food through the stomach, small intestine and colon in females with constipation.

As you read this form describing the study, ask any questions you have. Take your time to decide. Feel free to discuss the study with your family, friends, and healthcare provider before you decide. If you decide to participate, you may stop participating at any time during the study. You may decide not to participate. If so, none of your current benefits or normal health care will be affected in any way. When you feel comfortable that all your questions have been answered, and you wish to take part in this study, sign this form in order to begin your participation. Your signature means you have been told about the study and what the risks are. Your signature on this form also means that you want to take part in this study.

B. Number of Participants

The plan is to have 36 female participants with constipation take part in this study at Mayo Clinic.

C. Additional Information You Should Know

Albireo AB is funding the study. Albireo AB will pay your study doctor or the institution to cover costs related to running the study.

2. What Will Happen To You While You Are In This Research Study?

The study will last up to about forty-one (41) days from the date of the first visit, but the actual testing will be done over five (5) days on two (2) separate occasions in the Charlton 7 Clinical Research Unit (CRU).

If you agree to be in the study, you will be asked to participate in the following activities:

Visit 1 – You will be asked to report to the Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed. You will be asked to review and sign an informed consent form. You will have an interview with and a brief physical examination performed by a study physician. If you have not had a normal rectal exam to rule out a possible evacuation disorder documented within the past two (2) years, this exam will be performed at this time. You will have your height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) measured. You will be asked to have an electrocardiogram (ECG), a recording of the heart rhythm, performed. Blood and urine samples will be obtained to perform standard laboratory safety tests including hematology, chemistry, coagulation studies, urinalysis, urine screen for drugs of abuse and a urine pregnancy test for females of childbearing potential. You will be told the results of the pregnancy test. If it is positive, you will not be able to take part in the study Note that females who are status post bilateral tubal ligation, hysterectomy or postmenopausal do not need this test. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total volume of blood obtained for the standard laboratory tests is about two tablespoons. You will be asked to complete one (1) questionnaire, the long Bowel Disease questionnaire (BDQ) to confirm the diagnosis of constipation. This questionnaire requires about ten (10) to twenty (20) minutes total to complete. You will be given a bowel pattern diary and instructed in its completion. You will record numbers in this bowel pattern diary beginning today through Visit 7, the final day of the study for a maximum of 41 days. This visit will require approximately 45 to 75 minutes total.

During the study, you will be asked to refrain from donating blood; participating in other research studies or taking any additional over-the-counter or prescription medications or herbal supplements that have not been reviewed and approved by the physician or the study coordinator until the study has been completed.

Prior to Visit 2 - Females who may become pregnant must have a negative urine pregnancy test within forty-eight hours prior to receipt of radiation in the abbreviated transit scintigraphy test. You will be told the results of the pregnancy test. If it is positive, you will not be able to take part in the study. Females who have experienced a bilateral tubal ligation, hysterectomy or menopause will not need the pregnancy test. You may leave a urine sample at Desk Charlton 7 CRU the day prior to Visit 2 or bring in a sample with you should Visit 2 fall on a Monday.

Visit 2 – Within 7-14 days of Visit 1, you will be asked to return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to have your vital signs (pulse, blood pressure, respiration rate and temperature) measured. You will be given a capsule containing a small amount of radioactivity. This allows the movement of food particles through the colon to be measured using an external camera. You will be asked not to eat anything for the next two (2) hours and to return four (4) hours later for one five (5) minute camera picture. You will be asked to return the following morning at the same time you took the capsule for another five (5) minute camera image. If the camera indicates a state of constipation, you will continue in the study and receive the study medication. If the camera does not indicate a state of constipation, the study will be ended at this time.

Visit 3 – You will be asked to return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to have your vital signs (pulse, blood pressure, respiratiorn rate and temperature)

measured. You will be complete a six (6) question survey of constipation symptoms and treatment effectiveness before you receive the study medication. This survey will require less than five (5) minutes. You will be assigned to the study medication which is either a placebo (an inactive substance) or a 15 mg or 20 mg dose of A3309 study medication taken orally once daily for a total of fourteen (14) consecutive days, eleven (11) doses at home and three (3) at the Charlton CRU during the final transit scintigraphy test, Visit 4 to Visit 6. You will be put into one of these three (3) groups by chance (as in the flip of a coin). There is a two in three (2/3) chance that you would receive the A3309 study medication and a one in three (1/3) chance you would receive placebo. Neither you nor the research team will know what study medication you receive. However, in case of emergency, the physician would be able to find out. This study will require about fifteen minutes total.

Prior to Visit 4 - Females who may become pregnant must have a negative urine pregnancy test within forty-eight hours prior to receipt of radiation in the abbreviated transit scintigraphy test. You will be told the results of the pregnancy test. If it is positive, you will not be able to take part in the study. Females who have experienced a bilateral tubal ligation, hysterectomy or menopause will not need the pregnancy test. You may leave a urine sample at Desk Charlton 7 CRU the day prior to Visit 2 or bring in a sample with you should Visit 2 fall on a Monday.

Visit 4 - You will be asked to return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to have your vital signs (pulse, blood pressure, respiration rate and temperature) measured. A 12-lead ECG will be performed. You will be given the study medication and a capsule containing a small amount of radioactivity. This allows the movement of food particles through the colon to be measured using an external camera. About one hour after taking the capsule, you will stand in front of a special camera and an anterior and posterior image will be obtained. You will be given a scrambled egg breakfast meal; the eggs also contain a small amount of radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging procedure or the 'zero hour' image. Images will be obtained at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line (a small sterile plastic catheter) will be placed in your forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis (the amount of study medication present in the blood sample). Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360 and 480 minutes after the administration of the study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including hematology, chemistry, coagulation studies, and a urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about five (5) tablespoons. About four (4) hours after the breakfast, you will eat a standard chicken breast lunch meal. You will have camera images taken at five (5), six (6) and seven (7) hours. You will eat a standard roast beef sandwich snack, about eight (8) hours after the breakfast followed by a final camera image. These standard meals are not radioactive, they are supplied only so all participants in this study eat the same type and same amount of food this day. You will be asked not to eat or drink anything while you are having the transit test performed today other than what you are given. After the eight (8) hour image is completed, you will be instructed to return to the Charlton 7 CRU at specific times on the following two (2) mornings for administration of study medication and to obtain one five (5) minute camera image. This visit will require about nine to ten (9 to 10) hours total.

Visit 5 – The following morning**,** you will be asked to return to the Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed. You will receive the study medication and one five (5) minute camera image will be obtained. This visit will require fifteen (15) to twenty-five (25) minutes total.

Visit 6 – The following morning**,** you will be asked to return to the Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to receive the study medication. One five (5) minute camera image will be obtained. A study physician will perform a brif physical exam. You will be asked to complete a six (6) question survey of constipation symptoms and treatment effectiveness. This survey will require less than five (5) minutes. This visit will require fifteen (15) to twenty-five (25) minutes total.

Visit 7 – Within seven (7) to ten (10) days after completing Visit 6, you will return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink, to have your height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) measured. Blood and urine samples will be obtained to perform standard laboratory safety tests including hematology, chemistry, coagulation studies and a urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total amount of blood obtained for the standard laboratory tests is about two (2) tablespoons. A 12-lead ECG will be performed. You will be asked to turn in your completed bowel pattern diary. You will have a physical exam by the study doctor and an exit interview with study staff. This visit will require about twenty (20) to forty-five (45) minutes.

3. How Long Will You Be in This Research Study?

You will be in the study for a maximum of about forty-one (41) days.

4. Why You Might Want To Take Part In This Research Study

This study will not make your health better. It is for the benefit of research.

5. What Are the Risks Of This Research Study?

Data from two prior phase I studies using up to 5 mg in healthy volunteers and up to 10 mg in patients with functional constipation suggest that the study medication is well tolerated. In 26 normal healthy volunteers, the adverse events observed were: flatulence (27 percent), abdominal distension (19 percent), abdominal pain and nasopharyngitis (12 percent each), and diarrhea, nausea and headache, (2 percent each). In 25 constipation patients, the adverse events observed were: abdominal distension, nausea and headache (8 percent each).

As with any medication, allergic reactions are a possibility.

The risks of drawing blood include pain, bruising, or rarely, infection at the site of the needle stick.

You will be exposed to radiation in this study. The amount of radiation you will receive has a low risk of harmful effects.

Some questions you will be asked to answer in the study questionnaire(s) may make you feel uncomfortable. You may choose not to answer any questions that make you feel uncomfortable.

Pregnancy and Birth Control:

1) Will women of child-bearing-potential be allowed to participate in this study?
   Yes: Women of child-bearing-potential will be able to participate in this study if they have a negative pregnancy test and agree to use acceptable birth control (see # 4) since the risks to an unborn child are either unknown or potentially serious.

2) Will pregnant and/or nursing women be allowed to participate in this study?
   No: There is not enough medical information to know what the risks might be to an unborn child carried by a woman who takes part in this study.

3) Do you need to have a pregnancy test done to be part of the study?
   Yes: As part of this study a pregnancy test is required for all women who are able to become pregnant. A urine pregnancy test will be done. You will be told the results of the pregnancy test. If the pregnancy test is positive, you will not be able to take part in the study.

4) What types of birth control are acceptable?
   Surgical sterilization
   Approved hormonal contraceptives (such as birth control pills, Depo-Provera)
   Barrier methods (such as a condom or diaphragm) used with a spermicide
   An intrauterine device (IUD)
   Abstinence

Risk summary

Many side effects go away shortly after the A3309 study medication is stopped, but in some cases side effects can be serious, long lasting, or may never go away. Some side effects may not be known. Side effects may range from mild to life-threatening. Other drugs may be given to make side effects less serious and less uncomfortable. Talk to the researcher and/or your healthcare provider about side effects and ask any other questions.

6. What Other Choices Do You Have If You Don't Take Part In This Research Study?

This study is only being done to gather information. You may choose not to take part in this study.

7. Are There Reasons You Might Leave This Research Study Early?

Taking part in this research study is voluntary. You may decide to stop at any time. You should tell the researcher if you decide to stop and you will be advised whether any additional tests may need to be done for your safety.

In addition, the researchers, Albireo or Mayo may stop you from taking part in this study at any time:

- if it is in your best clinical interest,
- if you do not follow the study procedures ,
- if the study is stopped.

8. Will You Need To Pay For Any Of The Tests And Procedures?

You will not need to pay for tests and procedures which are done just for this research study. These tests and procedures are:

- Height, weight and vital signs
- Physical exams
- Urine pregnancy tests
- Laboratory tests including chemistry, hematology, coagulation studies, urinalysis and urine screen for drugs of abuse
- Electrocardiograms (ECGs)
- Blood draws for laboratory tests and pk samples
- Camera imaging studies
- Study medication (A3309 or placebo)
- Study meals

However, you and/or your health plan will need to pay for all other tests and procedures that you would normally have as part of your regular clinical care.

If you have study related questions regarding billing, insurance or reimbursement, stop by or call:
Admission and Business Services office, or call Patient Account Services at (507) 287-1819

9. Will You Be Paid For Participating In This Research Study?

If you finish the study, you will receive $600. This money is for the time you spend in this study. If you start the study but stop before finishing the study, you will receive part of this money.

10. What Happens If You Are Injured Or Ill Because You Were In This Research Study?

If you have side effects from taking part in this study, you need to report them to the researcher and your regular physician, and you will be treated as needed. Mayo will give medical services for treatment for any bad side effects from taking part in this study. Such services will be free if not covered by a health plan or insurance. No additional money will be offered.

If you have injuries directly resulting from the proper application of the study drug, whether at Mayo or another institution, the sponsor Albireo will pay for appropriate medical treatment beyond that covered by your health insurance or other third party or government programs, at no cost to you. Your study doctor can help you obtain this reimbursement. The sponsor will not be responsible for deductibles and co-pays. The sponsor Albireo will not offer to pay you for lost wages, disability, or discomfort due to any such side effects or injuries.

11. What Are Your Rights If You Are In This Research Study?

Taking part in this research study will not change your rights and benefits. Taking part in this research study does not give you any special privileges. If you decide to not participate in this study, or stop in the middle of the study, no benefits are taken away from you. Specifically, you do not have to be in this research study to receive or continue to receive medical care from Mayo Clinic.

You will be told of important new findings or any changes in the study or procedures that may affect you or your willingness to continue in the study.

12. What About Your Privacy?

Authorization To Use And Disclose Protected Health Information

Your privacy is important to us, and we want to protect it as much as possible. By signing this form, you authorize Mayo Clinic and the investigators to use and disclose any information created or collected in the course of your participation in this research protocol. This information might be in different places, including your original medical record, but we will only disclose information that is related to this research protocol for the purposes listed below.

This information will be given out for the proper monitoring of the study, checking the accuracy of study data, analyzing the study data, and other purposes necessary for the proper conduct and reporting of this study. If some of the information is reported in published medical journals or scientific discussions, it will be done in a way that does not directly identify you.

This information may be given to other researchers in this study, representatives of the company sponsoring the study, Albireo, including representatives in the USA or other countries, or private, state or federal government parties or regulatory authorities in the USA and other countries responsible for overseeing this research. These may include the Food and Drug Administration, the Office for Human Research Protections, or other offices within the Department of Health and Human Services, and the Mayo Clinic Office for Human Research Protections or other Mayo groups involved in protecting research subjects.

Information Disclosed to Study Sponsor

The study data sent by the study doctor to the sponsor does not include your name, address, social security number, or other information that directly identifies you. Instead, the study doctor assigns a code

number to the study data and may use your initials. Some study data sent to the sponsor may contain information that could be used (perhaps in combination with other information) to identify you (eg, date of birth). If you have questions about the specific health information that will be sent to the sponsor, you should ask the study doctor.

If this information is given out to anyone outside of Mayo, the information may no longer be protected by federal privacy regulations and may be given out by the person or entity that receives the information. However, Mayo will take steps to help other parties understand the need to keep this information confidential.

This authorization lasts until the end of the study. The study does not end until all data has been collected, checked (or audited) and analyzed. Sometimes this can be years after your study visits have ended. For example, this could happen if the results of the study are filed with a regulatory agency like the Food and Drug Administration

You may stop this authorization at any time by writing to the following address:

Mayo Clinic
Office for Human Research Protection
ATTN: Notice of Revocation of Authorization
200 1st Street SW
Rochester, MN 55905

If you stop authorization, Mayo may continue to use your information already collected as part of this study, but will not collect any new information.

12 a. Additional Information About Your Privacy

What Other Things Might the Sponsor do with Study Data?

In addition to the uses listed above, companies that sponsor studies often use study data for other purposes that are not part of the study. For example, the company might use the study data for research purposes to support the scientific objectives of the study described in this consent document, to learn more about the effects (good and bad) of any drug, device or treatment included in the study, to better understand the disease(s) included in the study, or to improve the design of future studies. Also, the company might share the study data with other companies it does business with. The company might do these things during the study, or after the study has ended, and would not have to ask for your permission to do so. The sponsor might still use study data, even after you stop your authorization, or the authorization expires, as long as the study data was collected before your authorization stopped or expired. The ways in which the study data could be used in the future may not be known now, so we can't give you the details.

13. What Will Happen to Your Samples?

Your samples obtained for pharmacokinetic measurements and for evaluation of biological effects of the drug will be sent to the sponsor or the sponsor's designee. The sponsor can use your samples solely for research purposes as described in the research study. Your sample will be returned to Mayo or destroyed

upon termination or expiration of the research study. Your sample will be sent to the sponsor in a coded format, which protects your identity. Mayo has the right to end storage of the sample without telling you Your samples obtained for standard safety assessments (chemistry, hematology, coagulation studies, urinalysis and urine screen for drugs of abuse) will be used as described for this study. When the study is done, they will be destroyed.

14. Who Can Answer Your Questions?

You can call …	At …	If you have questions or concerns about …
Principal Investigator: Michael Camilleri, MD **Study Coordinator**: Sanna McKinzie, MS	Phone: 6-2305 Phone: 6-8627	Questions about the study tests and procedures Research-related injuries or emergencies Any research-related concerns or complaints
Mayo Clinic IRB **Research Subject Advocate:** Shari Brumm	Phone: 507-266-4000 Toll-Free: 866-273-4681	Rights of a research subject Use of Protected Health Information Any research-related concerns or complaints
Research Billing	Rochester: 507-287-1819	Billing / Insurance Questions

15. Summary and Enrollment Signatures

You have been asked to take part in a research study, at Mayo Clinic. The information about this study has been provided to you to inform you about this study.

- I have read the whole consent form, and all of my questions have been answered to my satisfaction.
- I am satisfied that I have been given enough information about the purpose, methods, risks, and possible benefits of the study to decide if I want to join.
- I know that joining the study is voluntary and I agree to join the study.
- I know that I can call the investigator and research staff at any time with any questions or to tell them about side effects.
- I know that I may withdraw from the study at any time.

A copy of this form will be put in my medical records and I will be given a copy of this completed form.

Please sign and date to show that you have read all of the above guidelines. Please do not sign unless you have read this entire consent form. If you do not want to sign, you don't have to, but if you don't you cannot participate in this research study.

_____	_____	_____
(Date / Time)	(Printed Name of Participant)	(Clinic Number)

_____

(Signature of Participant)

_____	_____
(Date / Time)	(Printed Name of Individual Obtaining Consent)

_____

(Signature of Individual Obtaining Consent)

34.5 Patient bowel pattern diary

Daily Diary *Date* ☐☐☐☐☐☐

Please record the time the study medication was taken ____:____ am

If you had no bowel movements today, please check this box: ☐ ***no bowel movement today***

	Describe the consistency of bowel movement **1** Hard lumps **2** Lumpy sausage **3** Cracked sausage **4** Smooth sausage **5** Soft lumps **6** Mushy **7** watery	**Describe the ease of passage of bowel movement** **1** Manual Disimpaction **2** Enema needed **3** Straining needed **4** Normal **5** Urgent wo/pain **6** Urgent w/pain **7** Incontinent	**How much did you strain during the bowel movement?** **1** Not al all **2** A little bit **3** More straining than not **4** A great deal **5** An extreme amount	**Did you feel like you completely emptied your bowels?** **1** No **2** Yes	**Did you feel like you had a sensation of obstruction and/or blockage during the bowel movement?** **1** No **2** Yes
1 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
2 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
3 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
4 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
5 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
6 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
7 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐
8 ☐☐ hr. ☐☐ min ☐ am ☐ pm	☐	☐	☐	☐	☐

Have you had any unusual events today? **No** ☐ **Yes** ☐ *(complete below)*

Event _____ Mild / Moderate / Severe Resolved / Ongoing

Event _____ Mild / Moderate / Severe Resolved / Ongoing

Event _____ Mild / Moderate / Severe Resolved / Ongoing

Have you taken any atypical medications today? **No** ☐ **Yes** ☐ *(complete below)*

Medication _____	Medication _____	Medication _____
Dose _____	Dose _____	Dose _____
Time(s) _____	Time(s) _____	Time(s) _____

34.6 Survey of Constipation Symptoms and Treatment Efficacy

1. On average, how would you rate your constipation during the past 7 days?
   1 = None
   2 = Mild
   3 = Moderate
   4 = Severe
   5 = Very severe

2. Compared to before you started this study, how would you rate your constipation during the past 7 days?
   1 = Completely relieved
   2 = Considerably relieved
   3 = Somewhat relieved
   4 = Unchanged
   5 = Somewhat worse
   6 = Considerably worse
   7 = As bad as I can imagine

3. Compared to before you started this study, how would you rate the treatment effectiveness during the past 7 days?
   1 = Not at all effective
   2 = A little bit effective
   3 = Moderately effective
   4 = Quite a bit effective
   5 = Extremely effective

4. How would you rate your abdominal discomfort over the last 24 hours?
   1 = None
   2 = Mild
   3 = Moderate
   4 = Severe
   5 = Very severe

5. How would you rate your abdominal pain over the last 24 hours?
   1 = None
   2 = Mild
   3 = Moderate
   4 = Severe
   5 = Very severe

6. How would you rate your bloating over the last 24 hours?
   1 = None
   2 = Mild
   3 = Moderate
   4 = Severe
   5 = Very severe

Data and Safety Monitoring Plan

Mayo Clinic
CTSA – Research Resources

Protocol Application - Data and Safety Monitoring Plan Template (DSMP)

IRB #: 09-00618 **PI:** M. Camilleri, MD
Protocol Title: Effects of A3309, an Ileal Bile Acid Transport Inhibitor, on Small Bowel and Colonic Motor Functions in Female Patients with Functional Constipation
Person Completing This Form: S. McKinzie, MS
Date of Completion/Revision: 10/20/2009

Introduction
Please use the following template to develop or reflect your Data and Safety Monitoring Plan (DSMP). All protocols conducted on the Clinical Research Unit (CRU) or use any CTSA – Research Resources are required to have a DSMP.

Please include the DSMP with your protocol submission to the CTSA – Research Resources. The DSMP also requires IRB approval and may be submitted to the IRB with your new protocol at the time of submission or to the appropriate IRB review board with a modification form if the protocol is already IRB approved.

The specifics of the DSMP will depend on the nature, size, complexity, and risk of the research study. Your DSMP is one aspect of your protocol and the completed template is to be included with your protocol submission to both the CTSA – Research Resources and IRB.

The DSMP template includes the following steps:

1. Risk assessment
2. Data and Safety Monitoring Elements
   - Safety contact
   - Safety assessment (identify risks, assessment measures, individual doing assessments, frequency of assessments, and interventions to respond to risks)
   - Monitoring/reviewing of data and safety issues/trends (by whom and at what frequency)
   - Adverse event-grading and attribution
3. Implementation Plan
   - Reports, response to subject withdrawal/dropout, and decision making criteria for continuation, modification, or termination of protocol

Please contact one of the following for assistance with your DSMP:
Bettie Lechtenberg
CTSA – Research Resources
Administrative Office Manager/Protocol Specialist
Lechtenberg.bettie@mayo.edu

Eric L. Matteson, M.D.
CTSA – Research Resources
Research Resources Safety Committee Chair
Director of Research Subject Advocacy and Compliance
matteson.eric@mayo.edu

Protocol Application – Data and Safety Monitoring Plan Template

STEP 1: PROTOCOL RISK ASSESSMENT

A. Rank risks associated with participant characteristics **that may increase participant's vulnerability to unanticipated or undesired response (check all that apply).**

Category of Risk				
	Low	**Medium**	**High**	**Notes**
Age of Subjects	**x**			**Patients aged 18-65 only**
Childbearing Status	**x**			**Pregnant and breast-feeding females not allowed; use of contraception required; urine pregnancy tests required for females of child bearing potential**
Health/Disease Status	**x**			**Stable, otherwise healthy constipation patients**
Concurrent Stressors	**x**			**Not anticipated**
Communication Difficulties	**x**			**Not anticipated**
Cognitive Difficulties	**x**			**Not anticipated**
Other				

B. **Rank risks associated with procedures** (check all that apply).
Please note, procedural risks may increase when additional stimuli occurs prior to or during procedures. Risk may be related to the procedure's complexity, instrumentation, magnitude or interaction with other procedures.

Category of Risk					
	Minimal Risk	**Minor Increase over Minimal**	**More Than a Minor Increase over Minimal**	**Significant Risk**	**Notes**
Anthropomorphic evaluations	**x**				
Blood pressure measurement	**x**				**Vital signs**
Blood draws	**x**				**Safety labs x 3**
Electrocardiogram (EKG)	**x**				**Safety labs x 3**
GI transit study	**x**				**Low dose isotope**
*Investigational drug (IND)	**x**				**A3309, an IBAT**
Radiation	**x**				**Transit studies**
Venipuncture	**x**				**Safety labs x 3**
Venous lines	**x**				**pk sampling once**

Step 2: Data and Safety Monitoring Plan Elements

Method of data and safety monitoring:

Note: A Date and Safety Monitoring Board (DSMB) may be appropriate if any of the research procedures or participant characteristics are noted as "more than minor increase over minimal risk" and/or any of the items below are applicable to the study (mark all that apply).

☐ Phase I or II clinical trial	X Blinded (masked)
☐ Phase III clinical trial	X Randomized
☐ Multi-site	X Placebo-controlled

Questions:

1. Does this protocol require a DSMB? ☐ Yes X No

2. Who will provide the data and safety monitoring?

☐ An "external" DSMB funded by the protocol sponsor(s). **Complete Questions 3 and 4 below**.	X PI or designee. **Omit Question 3 and complete Question 4 below.**
☐ An "internal" DSMB or committee organized and funded by the investigator. **Complete Questions 3 and 4 below.**	☐ Other. Describe: _____
☐ No DSMB needed – Justification is attached.	

Identify who will review (monitor) data and safety assessment activities at Mayo (PI, Research Study Coordinator, etc.). [Specific safety assessment activities are listed in Section C.]

Name	Role on Project	Frequency of Review
Michael Camilleri, MD	PI	Monthly or as necessary for adverse events
Archana Rao, MD	CI	Monthly or as necessary for adverse events
Sanna McKinzie, MS	SC	As necessary for adverse events

Safety contact information

Who will be available on-call if an unexpected event including after-hour patient safety concerns would occur? At least one (1) person must be identified.

Name (degree/license)	Role on Project	Contact Information (telephone, pager, e-mail)
Michael Camilleri, MD	Staff Prinicipal Investigator	46218 pager 266-2305 secy
		camilleri.michael@mayo.edu
Archana Rao, MD	Fellow, available on call	127-13968
		rao.archana@mayo.edu

Safety Assessments – Risks and AEs (Please focus on level II, III, and primary procedures). See example below.

2.1 EXAMPLES

Example 1:

Risks and/or Anticipated Adverse Events	***Assessment measures***	***Individual doing assessment***	***Assessment intervals or frequency***	***Interventions to decrease or respond to risks***
a. Biopsy-				
Pain	*Subject communication*	*Physician, Study Coordinator, RN*	*At time of procedure, when subject leaves, and weekly by questionnaire.*	*Expected-Informed and support provided- Lidocane used at time of procedure and cataminophen for residual discomfort*
Bruising	*Subject Observation*	*Study Coordinator, RN*	*As above*	*Expected-Informed Decreased with pressure and ice*
Infection	*Subject symptoms/vital signs*	*Study Coordinator, RN*	*Participant instructed to call SC or PI*	*Performed by trained personnel*
b. Study drug-Hu-901	*See protocol, page 16.*	*Study Coordinator, RN*	*Daily review of lab reports and observe for 2 hours post infusion*	*Acetaminophen given for discomfort*

Example 2:

As a Level I/II risk protocol, monitoring will occur on the first three (3) subjects with future monitoring intervals determined and/or adjusted on the results of the first three (3) subjects.

Example 3:

We have a small, Level III risk protocol and will monitor all responses of the first three (3) subjects and every 1-2 subjects thereafter.

Please complete the following table and include risks and adverse events noted in the consent form, emphasizing those greater than minimal risk.

Risks and/or Anticipated Adverse Events	**Assessment measures**	**Individual doing assessment**	**Assessment intervals or frequency**	**Interventions to decrease or respond to risks**
Radiation exposure	Documentation of radiation exposure	GI technologists	Prior to dosing	Monthly monitoring of dosing log by PI
Bruising at IV or venipuncture or injection sites	Patient observation	CRU nurses, study staff	At time of procedure	Treatment with pressure and ice
Infection at IV or venipuncture or injection sites	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	Performed by trained personnel
Flatulence	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	None due to short half-life of medication
Abdominal bloating	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	None due to short half-life of medication
Abdominal pain	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	Assessment by study physician
Diarrhea	Patient	CRU nurses,	Patient instructed	Study physician

	observation	study staff	to contact study staff	assessment, advise regarding fluid intake, IV rehydration if severe
Nausea	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	Study physician assessment
Headache	Patient observation	CRU nurses, study staff	Patient instructed to contact study staff	Tylenol permitted, study physician assessment if severe

Note: Assessment findings are monitored by the individuals identified in Question #1.

D. Grading method for adverse event reporting

The PI must identify what scale will be used to grade adverse events (AEs) and indicate how the relationship between the adverse event and the protocol/intervention will be scaled. Each protocol may have a unique approach to grading AEs and the PI should consult the parent protocol and/or funding source for specific grading scales. Suggested guidelines for the grading of AEs are available below:

Example 1: Cancer Therapy Evaluation Program (CTEP) Common Toxicity Criteria (CTC II) available for viewing at http://ctep.info.nih.govi (see "Reporting Guidelines, Common Toxicity Criteria")

Example 2: Cancer Therapy Evaluation Program (CTEP) Common Terminology Criteria (CTC III)

Example 3: Common grading scale

0 No adverse event or within normal limits or not clinical significant
1 Mild - did not require treatment
2 Moderate - resolved with treatment
3 Severe - resulted in inability to carry on normal activities and required professional

Identify the scale used to grade the severity of adverse events in this study:

☐	AEs will be graded according to CTEP Common Toxicity Criteria (CTC II)
☐	AEs will be graded according to CTEP Common Terminology Criteria (CTC III)
☐	AEs will be graded according to the 0-5 scale shown above
X	AEs will be graded using another system (specify): AEs will be graded as per protocol (page 33) : **Mild** means awareness of symptoms or signs, but easily tolerated (acceptable) **Moderate** means discomfort enough to interfere with usual activity (disturbing)

	Severe means incapacity to work or to do usual activity (unacceptable)

Identify the attribution scale to be used in this study:

☐	The PI will determine the relationship of AEs to the test procedure/device/agent as definitely related, probably related, possibly related, unrelated, or unknown.
X	The PI will use an alternative attribution scale (specify): AEs will be attributed as per protocol (page 33): **Certain -** A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to drug administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (dechallenge) should be clinically plausible. The event must be definitive pharmacologically or phenomenologically, using a satisfactory rechallenge procedure if necessary. **Probable -** A clinical event - including laboratory test abnormality - with a reasonable time sequence to use of the product, unlikely to be attributed to concurrent disease or other drugs or chemicals, and which follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required. **Possible -** A clinical event - including laboratory test abnormality - with a reasonable time sequence to use of the product, but which could also be explained by concurrent disease or other drugs or chemicals. Information on drug withdrawal may be lacking or unclear. **Unlikely -** A clinical event - including laboratory test abnormality - with a temporal relationship with use of the product which makes a causal relationship improbable, and in which other drugs, chemicals or underlying disease provide plausible explanations. **Not related -** A clinical event - including laboratory test abnormality - with a temporal relationship with use of the product which makes a causal relationship impossible, and in which other drugs, chemicals or underlying disease provide a more probable explanations.

Step 3: Implementing the Data and Safety Monitoring Plan

A. Reporting of Adverse Events

B. Refer to the Mayo Institutional Review Board (IRB) web site for guidelines on reporting adverse events at (http://researchweb.mayo.edu/irb/index.html). Please check all reporting requirements that apply for this protocol:

☐ **National Institutes of Health (NIH)**	☐ **Food and Drug Administration (FDA)**
X IRB	**X CTSA – Research Resources**

X Sponsor (specify): Albireo

C. Subject withdrawals/dropouts

Describe how subject withdrawals/dropouts prior to study completion will be accommodated and reported. Include examples of reasons that may prompt subject withdrawal/dropout.

Description: Patient withdrawals or discontinuations prior to study completion will be described in an annual report to Mayo's IRB and copied to the CRU, FDA and sponsor. Reasons for withdrawal include abnormal laboratory test result, concomitant illness or withdrawal of consent by patient.

D. Decision making criteria – Stopping rules

Describe criteria for decision-making regarding continuation, modification, or termination of the clinical study (e.g. Review summarized data to determine response to procedure after 10% of subjects have been seen).

Description: A patient will be withdrawn from the study if she develops diarrhea severe enough to require IV rehydration. The study will be discontinued if greater than 40 percent of patients develop diarrhea severe enough to require IV rehydration.

E. Study meetings/discussions

Identify method for documenting study discussions and meeting minutes, as well as follow-up communications.

Method: Decisions that alter the study procedures or the human studies aspects will be communicated to the IRB, CRU and sponsor and the review minutes will be documented in the regulatory binder.

Please note:
All DSMP's are subject to ongoing review and modification as new information concerning data integrity and safety issues become available. Proposals for revision of DSMP's may come from any source, including the PI of the study, the study coordinator, CRU nursing staff, or the CTSA – Research Resources Program Director. Revisions of the DSMP may be expedited per the decision of the Program Director or the Research Resources Review Panel (RRRP) Chair but requires approval by the RRRP at its regular meeting.

34.7 Protocol amendment 1: List of changes

The protocol for the study is to be changed as follows:

Throughout the document links from ToC and to references as well as minor lay-out changes have been made and identified errors in the reference list have been corrected.

TITLE PAGE

Previous text:

Clinical Study Protocol	
Investigational Product	A3309
Study code	A3309-003
Protocol Version	1.2
Date	10 Nov 2009

Amendment No:	**Date of Amendment :**
Amendment No:	**Date of Amendment :**

Revised text:

Clinical Study Protocol	
Investigational Product	A3309
Study code	A3309-003
Protocol Version	1.3
Date	07 June 2010

Amendment No:1	**Date of Amendment :07 June 2010**
Amendment No:	**Date of Amendment :**

Reason for change:

Protocol Amendment 1 processing and administration.

(Please note that the Table of Contents has been updated to account for the amendment processing.)

PROTOCOL SYNOPSIS, Number of patients planned

Previous text:

Twelve completed female patients with functional constipation in each treatment group for a total of 36 patients.

Revised text:

Twelve ~~completed~~ female patients with functional constipation in each treatment group for a total of 36 patients.

Reason for change: Patients not completing the study has not been planned to be replaced

PROTOCOL SYNOPSIS, Methodology (2nd paragraph)

Previous text:

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than or equal to 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

Revised text:

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than ~~or equal to~~ 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

Reason for change: In the previous protocol different descriptions of this criterion appeared. In the amendment a consistent definition has been inserted throughout the protocol.

Section 7.3 Natural Laxation by Endogenous Bile Acids

Previous text:

Bile acids have a variety of physiologic functions and are actively reabsorbed (up to 95%) in the terminal ileum (5). Disruption of the enterohepatic circulation of bile acids due to ileal disease (e.g., Crohn's or radiation ileitis) or idiopathic bile acid malabsorption (BAM) causes chronic diarrhea [see systematic summary (6)]. Conjugated and non-conjugated bile acids induce secretion in the human colon (7,8) by activating intracellular secretory mechanisms [e.g. adenylate cyclase (9)] or by detergent effects resulting in mucus secretion (10), increased mucosal permeability (11) and inhibition of apical Cl-/OH- exchange (12). In addition, bile acids may induce propulsive contractions in canine [>20mM (13)] and human colon [1mM or 5mM (15)]. In humans, there is a relationship between the fecal bile acid excretion and colonic motility; however, this relationship is complicated by associated steatorrhea since the delivery of fatty acids to the colon may also accelerate colonic transit (16). Bile acid concentration in stool of patients with ileal resection may reach 21mM chenodeoxycholate [CDC (17)]. However, fecal concentrations of bile acids in diarrhea-predominant irritable bowel syndrome (IBS-D) or functional diarrhea are unknown, and up-regulation of the ileal active transporter (18) as a result of chronic loss of bile acids may reduce the bile acids reaching the colon. In studies of gallstone dissolution with 750-1000mg CDC per day (19), diarrhea occurred in 40% of patients. Therefore, an approach that results in the passage of higher concentrations of endogenous bile acids into the colon has the potential to provide a useful treatment for constipation and related conditions. Structure activity studies show that several primary and secondary bile acids are secretagogues in the mammalian colon.

Revised text:

Bile acids have a variety of physiologic functions and are actively reabsorbed (up to 95%) in the terminal ileum (5). Disruption of the enterohepatic circulation of bile acids due to ileal disease (e.g., Crohn's or radiation ileitis) or idiopathic bile acid malabsorption (BAM) causes chronic diarrhea [see systematic summary (6)]. Conjugated and non-conjugated bile acids induce secretion in the human colon (7,8) by activating intracellular secretory mechanisms [e.g. adenylate cyclase (9)] or by detergent effects resulting in mucus secretion (10), increased mucosal permeability (11) and inhibition of apical Cl-/OH- exchange (12). In addition, bile acids may induce propulsive contractions in canine [>20mM (13)] and human colon [1mM *(14)* or 5mM (15)]. In humans, there is a relationship between the fecal bile acid excretion and colonic motility; however, this relationship is complicated by associated steatorrhea *(15)* since the delivery of fatty acids to the colon may also accelerate colonic transit (16). Bile acid concentration in stool of patients with ileal resection may reach 21mM chenodeoxycholate [CDC (17)]. However, fecal concentrations of bile acids in diarrhea-predominant irritable bowel syndrome (IBS-D) or functional diarrhea are unknown, and up-regulation of the ileal active transporter (18) as a result of chronic loss of bile acids may reduce the bile acids reaching the colon. In studies of gallstone dissolution with 750-1000mg CDC per day (19), diarrhea occurred in 40% of patients *(20).* Therefore, an approach that results in the passage of higher concentrations of endogenous bile acids into the colon has the potential to provide a useful treatment for constipation and related conditions. Structure activity studies show that several primary and secondary bile acids are secretagogues in the mammalian colon *(11).*

Reason for change: Previous protocol had some inconsistencies in the literature references. This has now been corrected throughout the protocol

Section 8.1 Chemistry (5th paragraph)

Previous text:

Bile acids are mainly absorbed by a specific ileal bile acid transporter in the ileum (IBAT) and are returned to the liver, completing enterohepatic circulation (1). A3309 is a small molecule known to be an inhibitor of the IBAT that interrupts the enterohepatic circulation of bile acids by decreasing intestinal bile acid reabsorption, which results in an increased bile acid load to the colon. This stimulates increased fluid secretion in the colon and colonic motility; both effects are expected to benefit patients with constipation (2). Additionally, given the mode of action of A3309, beneficial effects on bile acid synthesis and cholesterol levels are anticipated (2,3). Bile acid reabsorption from the intestine is a very efficient process whereby 95% of the secreted bile acids are reabsorbed and IBAT, an integral brush border membrane glycoprotein that co-transports sodium and bile acid, appears to be a major regulator of the bile acid pool in animals and man. IBAT inhibitors prevent the reabsorption of bile acids from the ileum and their return to the liver. The liver compensates for this decrease in bile acid level by upregulating cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis(4,5).

Revised text:

Bile acids are mainly absorbed by a specific ileal bile acid transporter in the ileum (IBAT) and are returned to the liver, completing enterohepatic circulation (~~1~~*25*). A3309 is a small molecule known to be an inhibitor of the IBAT that interrupts the enterohepatic circulation of bile acids by decreasing intestinal bile acid reabsorption, which results in an increased bile acid load to the colon. This stimulates increased fluid secretion in the colon and colonic motility; both effects are expected to benefit patients with constipation (~~2~~*27*). Additionally, given the mode of action of A3309, beneficial effects on bile acid synthesis and cholesterol levels are anticipated (~~2,3~~*325*). Bile acid reabsorption from the intestine is a very efficient process whereby 95% of the secreted bile acids are reabsorbed and IBAT, an integral brush border membrane glycoprotein that co-transports sodium and bile acid, appears to be a major regulator of the bile acid pool in animals and man. IBAT inhibitors prevent the reabsorption of bile acids from the ileum and their return to the liver. The liver compensates for this decrease in bile acid level by upregulating cholesterol 7α-hydroxylase, the rate-limiting enzyme for bile acid synthesis(~~4,5~~*26,27*).

Reason for change: Previous protocol had some inconsistencies in the literature references. This has now been corrected throughout the protocol.

Section 11.1 Study Design (2nd paragraph)

Previous text:

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than or equal to 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

Revised text:

Within seven (7) to fourteen (14) days of Visit 1, eligible patients will return for an abbreviated scintigraphy test with images obtained only at 4 and 24 hours post In^{111} capsule ingestion. A geometric center at 24 hours must be less than ~~or equal to~~ 2.30, the median of normal colonic transit in normal healthy volunteers (as determined by analysis of a compilation of prior Camilleri studies), to qualify for randomization to study medication, either 15 or 20 mg A3309 or placebo administered orally once daily for fourteen (14) consecutive days. The allocation to treatment group will be concealed.

Reason for change: In the previous protocol different descriptions of this criterion appeared. In the amendment a consistent definition has been inserted throughout the protocol.

Section 11.2 Schedule of events

Previous text:

[1] A rectal exam to be performed to exclude evacuation disorder if a normal exam not documented within 2 years.

Revised text:

[1] A rectal exam to be performed *at Visit 1* to exclude *the possibility of an* evacuation disorder if a normal exam not documented within 2 years *by a qualified gastroenterologist. A patient may defer the rectal exam at Visits 6 and 7 if not clinically indicated.*

Reason for change: To ensure adequately trained physician and, as per clinical convention, rectal exam will only be performed based on clinical symptoms at visits 6 and 7.

Section 11.3 Schedule of Visits, Visit 1 Study Entry (2nd paragraph)

Previous text:

After reviewing and signing a patient ICF, they will have a brief interview (including demographics, medical history and current medications) and a physical examination performed by a study physician. If the results of a normal rectal examination to exclude the possibility of an evacuation disorder are not documented within the previous two years, this exam will be performed at this time. Height, weight and vital signs including temperature, pulse, blood pressure, and respiration rate will be obtained.

Revised text:

After reviewing and signing a patient ICF, they will have a brief interview (including demographics, medical history and current medications) and a physical examination performed by a study physician. If the results of a normal rectal examination to exclude the possibility of an evacuation disorder are not documented within the previous two years *by a qualified gastroenterologist*, this exam will be performed at this time. Height, weight and vital signs including temperature, pulse, blood pressure, and respiration rate will be obtained.

Reason for change: To ascertain that this exam is made by an appropriately trained physician.

Section 11.3 Schedule of Visits, Visit 4 Final Transit Scintigraphy and Pharmacokinetic Sampling

Previous text:

Patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses.
The study medication and a capsule containing a small amount of In^{111} radioactivity will be given. About one hour after taking the capsule, the first anterior and posterior images will be obtained. The patient will be given a scrambled egg breakfast meal; the eggs contain a small amount of Tc^{99} radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging procedure or the 'zero hour' image. Images will be obtained at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line will be placed in the forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis. Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360, 480 minutes after study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies, and UA. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about 75 mL. A 12-lead ECG will be performed. Four hours after the breakfast, a standard lunch meal consisting of chicken breast, baked potato and vanilla pudding is given. Images will be obtained at five, six and seven hours. Eight hours after the lunch, a standard dinner of a roast beef sandwich, sugar cookie and milk will be given followed by a final camera image. These standardized meals ensure that all participants in this study eat the same type and same amount of food this day. Patients are asked not to eat or drink anything other than these standardized meals.

Revised text:

Patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses.
The study medication and a capsule containing a small amount of In^{111} radioactivity will be given. About one hour after taking the capsule, the first anterior and posterior images will be obtained. The patient will be given a scrambled egg breakfast meal; the eggs contain a small amount of Tc^{99} radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging procedure or the 'zero hour' image. Images will be obtained at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line will be placed in the forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis. Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360, 480 minutes after study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies, and UA. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about 75 mL. A 12-lead ECG will be performed. *About f*~~F~~our hours after the breakfast, a standard *chicken breast* lunch meal ~~consisting of chicken breast, baked potato and vanilla pudding~~ is given. Images will be

obtained at five, six and seven hours. *About* ~~E~~*e*ight hours after the ~~lunch~~ breakfast, a standard ~~dinner of a~~ roast beef sandwich *snack*, ~~sugar cookie and milk~~ will be given followed by a final camera image. These standardized meals ensure that all participants in this study eat the same type and same amount of food this day. Patients are asked not to eat or drink anything other than these standardized meals.

Reason for change: To correct a fault in regard to description of food intake during the visit.

Section 11.3 Schedule of Visits, Visit 6 Transit Scintigraphy

Previous text:

The following morning**,** patients will return to the Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed, to receive the study medication followed by one five minute camera image. A brief physical exam will be performed by a study physician. Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses. Patients will complete a six question survey of constipation symptoms and treatment efficacy.

Revised text:

The following morning**,** patients will return to the Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed, to receive the study medication followed by one five minute camera image. A brief physical exam will be performed by a study physician. *Patients may defer the rectal exam if not clinically indicated.* Vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Patients will be asked about adverse events and concomitant medications by the study staff and the CRU nurses. Patients will complete a six question survey of constipation symptoms and treatment efficacy.

Reason for change: As per clinical convention, rectal exam will only be performed based on clinical symptoms at visits 6 and 7.

Section 11.3 Schedule of Visits, Visit 7 Study Completion

Previous text:

Within seven to ten days after Visit 6, patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Blood and urine samples will be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies and urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total amount of blood obtained for the standard laboratory tests is about 30 mL. A 12-lead ECG will be performed. The Bowel Pattern Diary will be turned in. A physical exam will be performed by the study doctor and an exit interview done with study staff.

Revised text:

Within seven to ten days after Visit 6, patients will return to Charlton 7 CRU fasting for the previous eight hours, no food or drink allowed. Height, weight and vital signs (pulse, blood pressure, respiration rate and temperature) will be measured. Blood and urine samples will be obtained to perform standard laboratory safety tests including a CBC, CMP, coagulation studies and urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) and one solitary pharmacokinetic sample. The total amount of blood obtained for the standard laboratory tests is about 30 mL. A 12-lead ECG will be performed. The Bowel Pattern Diary will be turned in. A physical exam will be performed by the study doctor*; the patients may defer the rectal exam if not clinically indicated* ~~and a~~. *A*n exit interview *will be* done with study staff.

Reason for change: As per clinical convention, rectal exam will only be performed based on clinical symptoms at visits 6 and 7.

Section 13 INCLUSION CRITERIA (#3)

Previous text:

3. A normal rectal exam result on file within the past 2 years or performed at screen to exclude the possibility of an evacuation disorder. Examination must exclude findings suggestive of an evacuation disorder such as high sphincter tone at rest, failure of perineal descent and spasm, tenderness or paradoxical contraction of the puborectalis muscles.

Revised text:

3. A normal rectal exam result on file within the past 2 years or performed at *Visit 1* screen *by a qualified gastroenterologist* to exclude the possibility of an evacuation disorder. Examination must exclude findings suggestive of an evacuation disorder such as high sphincter tone at rest, failure of perineal descent and spasm, tenderness or paradoxical contraction of the puborectalis muscles.

Reason for change: To ascertain that this exam is made by an appropriately trained physician either within the past two years or at Visit 1.

Section 14 EXCLUSION CRITERIA (#3)

Previous text:

3. Unable to withdraw all medications 48 hours prior to Visit 1; any medication that alters GI transit including but not limited to laxatives, magnesium or aluminum containing antacids, prokinetics, erythromycin, narcotics, anticholinergics, tricyclic antidepressants and SNRIs; analgesic drugs including opiates, NSAIDs and COX 2 inhibitors (Note: Tylenol is permitted), GABAergic agents and benzodiazepines.

   Note: All other concomitant medications will be reviewed on a case by case basis by the study physicians.

Revised text:

3. ~~Unable to withdraw all medications 48 hours prior to Visit 1; any medication that alters GI transit including but not limited to laxatives, magnesium or aluminum containing antacids, prokinetics, erythromycin, narcotics, anticholinergics, tricyclic antidepressants and SNRIs; analgesic drugs including opiates, NSAIDs and COX 2 inhibitors (Note: Tylenol is permitted), GABAergic agents and benzodiazepines.~~

   ~~Note:~~ ~~All other concomitant medications will be reviewed on a case by case basis by the study physicians.~~

   Taking any medication that in the opinion of the principal investigator has a potential to alter GI transit. This includes but is not limited to osmotic or stimulant laxatives, magnesium or aluminum-containing antacids, prokinetics, erythromycin, narcotics, anticholinergics, selective norepinephrine reuptake inhibitors (SNRIs), opiates, GABAergic agents and benzodiazepines.

   Note: *Selective serotonin reuptake inhibitor (SSRI) antidepressants are permissible at low, stable doses. Analgesics such as Tylenol, ibuprofen, naproxen and aspirin are permissible. All medications shall be reviewed by the principal investigator on a case by case basis.*

Reason for change: To ensure that the principal investigator is reviewing all medications. The list has been slightly modified based on current knowledge of potential for transit changes.

Section 19.1 Packaging, labeling and storage of Investigational Product (5th paragraph)

Previous text:

18 sachets containing two tablets each.
Content: A3309 tablets and/or Placebo tablets
Protocol Number: A3309-002
Patient no: XXXX
Storage: Room Temperature (preferably 20 – 25˚C)
Batch No: 96202-YYMM-XX
Use by: YYYY-MM-DD
Caution: New drug - Limited by Federal (USA) Law to Investigational Use
Sponsor: Albireo AB, Arvid Wallgrens backe 20, SE-413 46 Gothenburg, Sweden
Manufactured by: Galenica AB, Medeon, SE-205 12 Malmoe, Sweden
Clinical site: Mayo Clinics, xxxxxxxxxxx, phone: xxxxxx
Keep Out of Reach of Children.
Take both tablets from one sachet orally daily before breakfast.

Revised text:

18 sachets containing two tablets each.
Content: A3309 tablets and/or Placebo tablets
Protocol Number: A3309-00~~2~~*3*
Patient no: XXXX
Storage: Room Temperature (preferably 20 – 25˚C)

Batch No: 96202-YYMM-XX
Use by: YYYY-MM-DD
Caution: New drug - Limited by Federal (USA) Law to Investigational Use
Sponsor: Albireo AB, Arvid Wallgrens backe 20, SE-413 46 Gothenburg, Sweden
Manufactured by: Galenica AB, Medeon, SE-205 12 Malmoe, Sweden
Clinical site: Mayo Clinics, xxxxxxxxxxx, phone: xxxxxx
Keep Out of Reach of Children.
Take both tablets from one sachet orally daily before breakfast.

Reason for change: Correction of typographic error

Section 19.2 Doses and treatment regimens (3rd bullet)

Previous text:

- Evaluation of the results of the A3309-001 study showed:
    - A minor signal on C4 and on lipid parameters observed at the 3 mg and the 10 mg dose levels produced a stronger signal;
    - Efficacy signals (colon transit, stool form scale, and frequency of BMs) evident in the 10 mg dose group. However, no diarrhea or incapacitating "very loose stools" were observed in the 10 mg dose group.

Revised text:

- Evaluation of the results of the A3309-001 study showed:
    - A minor signal on C4 and on lipid parameters observed at the 3 mg and the 10 mg dose levels produced a stronger signal;
    - Efficacy signals (colon transit, stool form scale (*28*), and frequency of BMs) evident in the 10 mg dose group. However, no diarrhea or incapacitating "very loose stools" were observed in the 10 mg dose group.

Reason for change: Update of reference list

Section 21.2 Clinical efficacy assessment (#1 2nd paragraph)

Previous text:

Relative to the time of consumption of breakfast meal, abdominal camera images are initially obtained every 30 minutes for the first two hours, then hourly for the next six hours. At 4 hours, a standard lunch (chicken breast, potato, pudding and milk, the "Stanghellini" meal, 550 kcal) will be given. Camera images will be taken at 5, 6, and at 8 hours post breakfast meal. At 8 hours, the standard dinner (roast beef sandwich, sugar cookie and milk, the "Greydanus" meal, 750 kcal) will be given. The patient will leave the CRU after the completion of the 8 hour image and will return the following two mornings in order to receive study medication and obtain 24 and 48 hour scans fifteen minutes later. The performance characteristics of this test are summarized elsewhere. Please see relevant references on the methods (6,7,8,9,10).

Revised text:

Relative to the time of consumption of breakfast meal, abdominal camera images are initially obtained every 30 minutes for the first two hours, then hourly for the next six hours. At *about* 4 hours, a standard *chicken breast* lunch ~~(chicken breast, potato, pudding and milk, the "Stanghellini" meal, 550 kcal)~~ will be given. Camera images will be taken at 5, 6, and at 8 hours post breakfast meal. At *about* 8 hours, the standard ~~dinner (~~roast beef sandwich *snack*, ~~sugar cookie and milk, the "Greydanus" meal, 750 kcal)~~ will be given. The patient will leave the CRU after the completion of the 8 hour image and will return the following two mornings in order to receive study medication and obtain 24 and 48 hour scans fifteen minutes later. The performance characteristics of this test are summarized elsewhere. Please see relevant references on the methods (~~6,7,8,9,10~~ *29,30*).

Reason for change: To correct a fault in regard to description of food intake during the visit. Update of reference list.

Section 24.2 Analysis data sets

Previous text:

The primary analyses will follow the intent to treat (ITT) paradigm with all patients randomized included in the analyses. Those patients with missing response values will have their missing values imputed via the overall (patients with non-missing data) mean and a corresponding adjustment in the ANCOVA residual error variance degrees of freedom (subtracting one for each missing value imputed).

Safety data will be presented for all patients receiving investigational product.

Revised text:

The primary analyses will follow the intent to treat (ITT) paradigm with all patients randomized included in the analyses. Those patients with missing response values will have their missing values imputed via the overall (patients with non-missing data) mean and a corresponding adjustment in the ANCOVA residual error variance degrees of freedom (subtracting one for each missing value imputed). *A per protocol analysis of transit data will also be performed. The per protocol population consists of all patients having at least 80 % compliance with study drug and having participated in the transit evaluation.*

Safety data will be presented for all patients receiving investigational product.

Reason for change: To add an analysis comprising patients having fully completed the transit evaluation without imputing any data as per the ITT paradigm.

Section 24.6 Stool frequency and stool consistency

Previous text:

Patients are asked to keep a daily bowel pattern diary documenting the number of stools and the consistency (Bristol Stool Scale) of each stool. These are averaged as number of bowel movements or consistency of bowel movements per day per patient. Number of bowel movements and consistency are subsequently averaged over the study periods (i.e. run-in and treatment) per patient. The ANCOVA will be used to assess treatment effects on each of these two measures separately, using the corresponding run-in values as covariates. In addition, treatment effects on specific constipation symptoms , considered as discrete categorical responses, will be evaluated by a contingency table analysis of the post-treatment responses based on the final post-treatment bowel pattern diaries and questionnaires concerning constipation symptoms and treatment efficacy.

Revised text:

Patients are asked to keep a daily bowel pattern diary documenting the number of stools and the consistency (Bristol Stool Scale(*28*)) of each stool. These are averaged as number of bowel movements or consistency of bowel movements per day per patient. Number of bowel movements and consistency are subsequently averaged over the study periods (i.e. run-in and treatment) per patient. The ANCOVA will be used to assess treatment effects on each of these two measures separately, using the corresponding run-in values as covariates. In addition, treatment effects on specific constipation symptoms , considered as discrete categorical responses, will be evaluated by a contingency table analysis of the post-treatment responses based on the final post-treatment bowel pattern diaries and questionnaires concerning constipation symptoms and treatment efficacy.

Reason for change: Update of reference list

Section 24.9 Assessment of sample size

Previous text:

† * Based on data from previous studies(11,12,13,14,15,16) using similar methods to those proposed here. Transit data† from C-IBS studies (e.g. Renzapride, prucalopride, Tegaserod).

Revised text:

† * Based on data from previous studies(~~11,12,13,14,15,16~~ *31,32,33*) using similar methods to those proposed here. Transit data† from C-IBS studies (e.g. Renzapride, prucalopride, Tegaserod).

Reason for change: Update of reference list

33 REFERENCES

Previous text:

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[6] Burton DD, Camilleri M, Mullan BP, Forstrom LA, Hung JC. Colonic transit scintigraphy labeled activated charcoal compared with ion exchange pellets. J Nucl Med 1997;38:1807-1810.

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Reason for change: Complete revision of reference list given errors in the list in the initial protocol

Section 34.1 Study Flow Chart

Previous text:

[1] A rectal exam to be performed to exclude evacuation disorder if a normal exam not documented within 2 years.

Revised text:

[1] A rectal exam to be performed *at Visit 1* to exclude *the possibility of an* evacuation disorder if a normal exam not documented within 2 years *by a qualified gastroenterologist. A patient may defer the rectal exam at Visits 6 and 7 if not clinically indicated.*

Reason for change: To ensure appropriate staff and, as per clinical convention, rectal exam will only be performed based on clinical symptoms at visits 6 and 7.

Section 34.4 Patient informed consent form, 2. What Will Happen To You While You Are In This Study?, Visit 4.

Previous text:

Visit 4 - You will be asked to return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to have your vital signs (pulse, blood pressure, respiration rate and temperature) measured. A 12-lead ECG will be performed. You will be given the study medication and a capsule containing a small amount of radioactivity. This allows the movement of food particles through the colon to be measured using an external camera. About one hour after taking the capsule, you will stand in front of a special camera and an anterior and posterior image will be obtained. You will be given a scrambled egg breakfast meal; the eggs also contain a small amount of radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging procedure or the 'zero hour' image. Images will be obtained

at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line (a small sterile plastic catheter) will be placed in your forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis (the amount of study medication present in the blood sample). Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360 and 480 minutes after the administration of the study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including hematology, chemistry, coagulation studies, and a urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about five (5) tablespoons. Four (4) hours after the breakfast, you will eat a standard lunch meal consisting of chicken breast, baked potato, and vanilla pudding. You will have camera images taken at five (5), six (6) and seven (7) hours and eat a standard dinner of a roast beef sandwich, sugar cookie and milk eight (8) hours after the breakfast followed by a final camera image. These standard meals are not radioactive, they are supplied only so all participants in this study eat the same type and same amount of food this day. You will be asked not to eat or drink anything while you are having the transit test performed today other than what you are given. After the eight (8) hour image is completed, you will be instructed to return to the Charlton 7 CRU at specific times on the following two (2) mornings for administration of study medication and to obtain one five (5) minute camera image. This visit will require about nine to ten (9 to 10) hours total.

Revised text:

Visit 4 - You will be asked to return to Charlton 7 CRU fasting for the previous eight (8) hours, no food or drink allowed, to have your vital signs (pulse, blood pressure, respiration rate and temperature) measured. A 12-lead ECG will be performed. You will be given the study medication and a capsule containing a small amount of radioactivity. This allows the movement of food particles through the colon to be measured using an external camera. About one hour after taking the capsule, you will stand in front of a special camera and an anterior and posterior image will be obtained. You will be given a scrambled egg breakfast meal; the eggs also contain a small amount of radioactivity. Images will be obtained immediately after the egg breakfast; this image is considered the start of the imaging procedure or the 'zero hour' image. Images will be obtained at 30, 60 and 90 minutes later and at 2, 3, 4, 5, 6, 7, and 8 hours after the 'zero hour' image. An intravenous line (a small sterile plastic catheter) will be placed in your forearm or hand to obtain small volume blood samples for pharmacokinetic (pk) analysis (the amount of study medication present in the blood sample). Small volume blood samples will be drawn immediately before the administration of the study medication and at 30, 60, 90, 120, 180, 240, 360 and 480 minutes after the administration of the study medication. Blood and urine samples will also be obtained to perform standard laboratory safety tests including hematology, chemistry, coagulation studies, and a urinalysis. Blood samples will also be obtained for pharmacodynamics (C4, total cholesterol, LDL, HDL, and triglycerides) at this time. The total amount of blood taken will be about five (5) tablespoons. *About* ~~*F*~~*four* (4) hours after the breakfast, you will eat a standard ~~lunch meal consisting of~~ chicken breast~~, baked potato, and vanilla pudding~~ *lunch meal*. You will have camera images taken at five (5), six (6) and seven (7) hours. *You will* ~~and~~ eat a standard ~~dinner of a~~ roast beef sandwich~~, sugar cookie and milk~~ *snack about* eight (8) hours after the breakfast followed by a final camera image. These standard meals are not radioactive, they are supplied only so all participants in this study eat the same type and same amount of food this day. You will be asked not to eat or drink anything while you are having the transit test performed today other than what you are given. After the eight (8) hour image is completed, you will be instructed to return to the Charlton 7 CRU at specific times on the following two (2) mornings for administration of study medication and to obtain one five (5) minute camera image. This visit will require about nine to ten (9 to 10) hours total.

Reason for change: To correct a fault in regard to description of food intake during the visit.