TITLE PAGE

Protocol Title: A Phase I, First Time in Human, Two Part, Randomized, Placebo-Controlled, Double-Blind (Sponsor Unblind), Single and Repeat Dose Escalating Study to Evaluate the Safety, Tolerability and Pharmacokinetics of GSK3352589, a REarranged during Transfection (RET) Growth Factor Receptor Tyrosine Kinase Inhibitor, in Normal Healthy Volunteers

Protocol Number: 207440

Short Title: A Phase 1, First Time in Human Study to Evaluate GSK3352589, a REarranged during Transfection (RET) Growth Factor Receptor Tyrosine Kinase Inhibitor, in Normal Healthy Volunteers

Compound Number: GSK3352589

Sponsor Name and Legal Registered Address:

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1. SYNOPSIS

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Rationale:

Irritable bowel syndrome (IBS) is a relatively common gastrointestinal (GI) illness with a prevalence of approximately 5 to 20% and characterized by a constellation of clinical symptoms including abdominal pain and discomfort, abnormal bowel habits, and bloating [Camilleri, 2012] [Halland, 2013] [Longstreth, 2006]. Because the etiology of the disease has not been established, diagnosis is difficult and relies primarily on the presence of a specific symptom complex occurring with a minimum frequency in the absence of an alternative explanation. It is generally believed that the sensory inputs/outputs in the peripheral and central nervous system are altered in such a way that a patient with IBS has a heightened and disproportionate sensory experience for a given stimulus, i.e. visceral hypersensitivity [Azpiroz, 2007]. Visceral hypersensitivity may result from increased nerve fiber density and sprouting in the intestinal mucosal tissues of patients with IBS [Dothel, 2015].

REarranged during Transfection (RET) is a neuronal growth factor receptor tyrosine kinase whose activity is critical for the development of the enteric nervous system (ENS), the kidney and spermatogenesis [Airaksinen, 2002]. While its role during the development of the ENS has been well established, recent data strongly implicate RET kinase in the maintenance and plasticity of the adult ENS. By reducing RET signaling, RET kinase inhibition may modulate sensory neuron activity in the enteric nervous system and ameliorate visceral hypersensitivity. Preliminary *in vitro* and *in vivo* studies suggest that RET kinase inhibition is able to attenuate the function of cholinergic neurons. Taken together, these results suggest that treatment of IBS patients with a RET kinase inhibitor has the potential to reduce abdominal pain and discomfort in patients with IBS via a novel mechanism of action which may normalize both visceral hypersensitivity and abnormal motility by inhibiting the function of cholinergic neurons. Thus, the patient population that may derive the greatest benefit would consist of individuals with IBS-diarrhea (IBS-D).

GSK3352589 is a potent and selective inhibitor of RET kinase. The current study is designed to assess the safety, tolerability and PK of escalating single and repeat oral doses of GSK3352589 in normal healthy volunteers. This study is the first administration of GSK3352589 to humans.

Objectives and Endpoints:

Objective	Endpoint
Primary	
Part A Single Dose • To evaluate the safety and tolerability of single escalating oral doses of GSK3352589 administered in the fasted state and a single dose of GSK3352589 administered in the fed state in healthy adult subjects • To evaluate the pharmacokinetic parameters of escalating single oral doses of GSK3352589 under fasting and fed (for one dose only) conditions in healthy adult subjects **Part B Repeat Dose** • To evaluate the safety and tolerability of repeat escalating oral doses of GSK3352589 administered once or twice daily in the fasted or fed state • To characterize the PK of GSK3352589 of repeat escalating oral doses administered once or twice daily in the fasted or fed state	• Clinical safety and tolerability data including spontaneous adverse event (AE) reporting, clinical observations, physical examination findings, 12-lead electrocardiograms (ECG), vital signs, Bristol Stool Form Scores (BSFS), clinical laboratory tests. • PK parameters obtained following administration of GSK3353589. PK parameters include areas under the plasma concentration versus time curve, AUC over the dosing interval (and other time intervals), Cmax, Tmax, and terminal half life (t ½), for Days 1 and 14 will be analyzed as data permit.
Exploratory	
• To explore the effect of GSK3352589 on the postprandial plasma profiles of total glucagon like peptide-1 (GLP-1) and total peptide YY (PYY) following repeat oral doses of GSK3352589 administered once or twice daily	• Postprandial challenge (mixed-meal) profiles of total GLP-1 and total PYY.
• To investigate the biotransformation of GSK3352589 in plasma and urine	• Provide samples of plasma and urine for the identification of any compound-derived metabolite(s).

Overall Design:

This randomized, double-blind (sponsor unblinded), placebo controlled, dose escalation study will be divided into two parts: Part A is a single dose escalating, four period crossover (Cohort 1) with a two-period, single sequence pilot food effect study (Cohort 2) and Part B is a 14 day repeat dose escalating, ascending cohort study.

The study will be composed of 3 periods for all subjects (Screening, Treatment, and Follow-up). Subjects will participate in either Part A or Part B. A subject's total time involved in the study will be approximately 10 weeks for subjects enrolled in Part A, Cohort 1 and approximately 8 weeks for subjects enrolled in Part A, Cohort 2. Subjects enrolled in Part B will be involved in the study for a total time of approximately 7 weeks.

All dosing periods for subjects participating in Part A will be completed before beginning enrollment in Part B.

Part A:

Number of Participants: A sufficient number of healthy volunteers (males and women of non-childbearing potential) will be screened to enroll 16 subjects (8 subjects/cohort).

Treatment Period and Duration: The planned dose range for Cohort 1 is single doses of 2 to 400 mg in the fasted state. The actual doses to be administered may be adjusted based on safety, tolerability and PK data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose for Cohort 1. Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits. A single dose of GSK33352589 will be administered in the fasted and fed states in Cohort 2. The dose selected to evaluate the food effect will allow for a 2X increase in bioavailability and predicted exposures will not exceed the highest observed exposures at prior doses.

Cohort 1 (Single Dose Escalation[1])

Treatment Sequence	Subjects	Period 1	Period 2	Period 3	Period 4
A	PPD and PPD	Placebo	D2	D3	D4
B	and	D1	Placebo	D3	D4
C	and	D1	D2	Placebo	D4
D	and	D1	D2	D3	Placebo

1 Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits.

Cohort 2 (Pilot Food Effect)

Treatment Sequence	Subjects	Period 1	Period 2
E	PPD	D5	D5 + food
F	PPD and PPD	Placebo	Placebo + food

Part B:

Number of Participants: Up to six (6) cohorts are planned and will be conducted sequentially. A sufficient number of healthy volunteers (males and women of non-childbearing potential) will be screened to randomize 48 subjects (8 subjects/cohort; 6 subjects will be randomized to GSK3352589 and 2 subjects will be randomized to placebo).

Treatment Period and Duration: The planned dose range is 2 mg once daily to 200 mg twice daily administered for 14 days. Selection of the starting dose for Part B, along with requirements for fasted or fed state study drug administration, will occur after review of the safety, tolerability and PK data of all subjects enrolled in Part A. The actual doses to be administered may be adjusted based on safety, tolerability and PK data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose. Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits.

2. SCHEDULE OF ACTIVITIES (SOA)

Part A: Cohort 1 and 2 – Single Dose – Screening and Day 1

	Screening	Day -1	Day 1											
	Screen (within 21d)	Day -1[1]	Pre-dose	0h	0.5h	1h	1.5h	2h	2.5h	3h	4h	5h	8h	12h
Informed Consent	X													
Inclusion and exclusion criteria	X													
Demography	X													
Medical/medication/drug/alcohol history	X	X[2]												
Full Physical Exam (Inc Wt, Ht)	X	X[2]												
Vital Signs (BP, HR, Temperature)[3]	X	X	X			X					X			X
12-Lead ECG	X		X			X					X			X
Safety Labs (Chemistry, Hematology, and Urinalysis)	X	X												
Pregnancy Test[4]	X	X												
Estradiol/FSH[5]	X													
Urine Drug Screen, Alcohol Screen & Cotinine[6]	X	X												
HIV, Hep B, Hep C	X													
Administration of mixed meal breakfast[7]				X										
Dosing				X										

	Screening	**Day -1**	**Day 1**											
	Screen (within 21d)	**Day -1**[1]	**Pre-dose**	**0h**	**0.5h**	**1h**	**1.5h**	**2h**	**2.5h**	**3h**	**4h**	**5h**	**8h**	**12h**
Plasma PK Sampling[8]			X		X	X	X	X	X	X	X	X	X	X
Bristol Stool Form Scale (BSFS)[9]		←==========→												
Concomitant Medication			←==========→											
AE Assessment			←==========→											
SAE Assessment	←==========→													
Outpatient Visits	X													
Inpatient Check-in		X												

1. Within each cohort, subjects will return for their next scheduled dosing period approximately 14 days after administration of the study drug during the prior dosing period
2. Period 1 Day -1: Update medical/ medication history; No Wt/ Ht at Day-1.
3. Full vital signs to be taken at screening and Day-1, and on Day 1 predose. Blood pressure and heart rate will be taken 1 hr, 4 hr and 12 hr after administration of study drug on Day 1.
4. Serum hCG at screening, urine hCG pre-dose on Day -1 – all females.
5. If indicated
6. An alcohol breath test is acceptable.
7. Mixed meal breakfast ONLY for Cohort 2, Period 2 immediately following dosing.
8. PK will be collected on Day 1, 2 and 3 with time relative to Day 1 dose. Day 1 at predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12 hrs.
9. BSFS for all stool samples beginning Day -1.

Part A: Cohort 1 and 2 – Single Dose – Days 2-3 and Follow Up

	Day 2	**Day 2**	**Day 2**	**Day 3**	**Follow Up[1]**
	16h	24h	36h	48h	**(Days 7-10)**
Medical/medication/drug/alcohol history					
Brief Physical Exam				X[2]	X[2]
Vital Signs (BP, HR, Temperature)		X		X	X
12-Lead ECG		X			X
Safety Labs (Chemistry, Hematology, and Urinalysis)				X	X
Pregnancy Test[3]					X
Plasma Pharmacokinetic Sampling[4]	X	X	X	X	
BSFS[5]	←==========			==========→	
Concomitant Medication	←==========			==========→	
AE Assessment	←==========			==========→	
SAE Assessment	←==========			==========→	
Outpatient Visits					X
Inpatient Check-out				X	

1. Subjects will return approximately 1 week after check out from their last actual dosing period (i.e. Day 1 of Dosing Period 3) for a final follow up visit. If a subject discontinues from the study prior to completing all 4 periods in Cohort 1 or all 2 periods in Cohort 2, they will complete their early termination visit 7-10 days after their last dose received.
2. No Wt/ Ht, Brief, symptom directed Physical Exam.
3. Serum hCG at follow up – all females.
4. PK will be collected on Days 1, 2 and 3 with time relative to Day 1 dose. Day 2 at: 16, 24, & 36 hrs & Day 3 at 48 hrs.
5. BSFS for all stool samples beginning Day -1

Part B – Repeat Dose – Fasted (QD & BID)

	Screening Period and Baseline Assessments (within 21 days)			**Treatment Period**							**Follow Up Period or Early Termination**
	Screening	**Day -2**	**Day -1**	**Day 1**	**Days 2-6**	**Day 7**	**Day 8-12**	**Day 13**	**Day 14**	**Day 15**	**Day 22-25**
Informed Consent	X										
Inclusion and exclusion criteria	X	X[1]									
Demography	X										
Medical/surgical/alcohol history	X	X[1]									
Full Physical Exam (Inc Wt, Ht)	X	X[1]				X[1]				X[1]	X[1]
Vital Signs (BP, HR, Temperature) [2]	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG[3]	X			X		X			X		X
Safety Labs (Chemistry, Hematology, and Urinalysis)	X	X				X				X	X
Pregnancy Test[4]	X	X									X
Estradiol/FSH[5]	X										
Cotinine Test, Urine Drug Screen & Alcohol Screen[6]	X	X									
HIV, Hep B, Hep C	X										
Administration of standard meal at dinner		X	X					X			
Administration of mixed meal at breakfast[7]			X	X		X			X		

	Screening Period and Baseline Assessments (within 21 days)			**Treatment Period**							**Follow Up Period or Early Termination**
	Screening	**Day -2**	**Day -1**	**Day 1**	**Days 2-6**	**Day 7**	**Day 8-12**	**Day 13**	**Day 14**	**Day 15**	**Day 22-25**
Pharmacodynamic testing[8]			X	X					X		
Dosing[9]				X	X	X	X	X	X		
Plasma PK Sampling[10]				X	X	X	X		X	X	
Plasma Metabolite Sampling[11]				X					X		
Urinary PK &Metabolite Sampling[12]				X					X		
Bristol Stool Form Scale (BSFS)[13]		←==========									
Prior and Concomitant Medication	←==========										
AE Assessment				←==========							
SAE Assessment	←==========										
Outpatient Visits	X										X
Inpatient Check-in		X									
Inpatient Check-out										X	

1. Day -2: Confirm eligibility, update medical / medication history. Ht at Screening only. Wt at screening and on Days -1, 7 and 15. Brief symptom directed physical exam on Days 7, 15 and at FU.
2. Full vital signs will be taken at screening, D-2, D-1, Day 1, 7 and 14 predose, D15, & FU. Vital signs on Day 1, 7 and 14 will be taken 4 hrs after admin of study drug.
3. 12 lead ECG will be on Days 1, 7 and 14 predose and approximately 4 hours after administration of study drug.
4. Serum hCG at screening and follow up, urine hCG pre-dose on Day -2 – all females.
5. If indicated
6. An alcohol breath test is acceptable.
7. Mixed meal breakfast to be eaten on days -1, 1, 7 and 14 at approximately 2 hours post dose and eaten within 15 minutes.

8. Blood samples for PD on **Day -1, Day 1 and Day 14** at the following timepoints: 15, 10, and 5 minutes prior to breakfast, and 0.5, 1, 2, and 4 hrs post breakfast. Lunch to be eaten after 4 hr sample.
9. **QD Dosing** will be at approximately 0800 hours. **BID Dosing** will be at approximately 0800 and PM dose to be administered 10 hours following AM dose.
10. **For QD dosing**: **Days 1 and 14** PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, and 24 hrs (within 10 minutes or less prior to next dose).
    For QD dosing, **Day 7**: PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 1, 2, 4, 8, and 24 hours (10 minutes prior to next dose on Day 8).
    For BID dosing: **Days 1 and 14** PK blood samples will be obtained predose within 10 minutes prior to 1st dose and then postdose at the following timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 10 (10 mins prior to 2nd dose), 11, 12, 14, 16, and 24 hrs (within 10 minutes or less prior to next dose).
    For BID dosing, **Day 7**: PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 1, 2, 4, 8, 10 hours (10 minutes or less prior to 2nd dose) and 24 hours (10 minutes prior to next dose on Day 8).
11. Plasma samples for metabolite profiling will be collected pre-dose and at all PK timepoints post-dose (for 0-24 hours for all cohorts, however only samples from the highest dose cohort will be analyzed.
12. Urine samples to aid metabolite structure identification will be collected pre-dose and over 0-24 hours for all cohorts and analyzed for the highest dose cohort samples only
13. BSFS for all stool samples beginning with time of check into clinical facility.

Part B – Repeat Dose – Fed (QD and BID)

	Screening Period and Baseline Assessments (within 21 days)			**Treatment Period**							**Follow Up Period or Early Termination**
	Screening	**Day -2**	**Day -1**	**Day 1**	**Days 2-6**	**Day 7**	**Day 8-12**	**Day 13**	**Day 14**	**Day 15**	**Day 22-25**
Informed Consent	X										
Inclusion and exclusion criteria	X	X[1]									
Demography	X										
Medical/surgical/alcohol history	X	X[1]								X[1]	X[1]
Full Physical Exam (Inc Wt, Ht)	X	X[1]				X[1]				X[1]	X[1]
Vital Signs (BP, HR, Temperature) [2]	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG[3]	X			X		X			X		X
Safety Labs (Chemistry, Hematology, and Urinalysis)	X	X				X				X	X
Pregnancy Test[4]	X	X									X
Estradiol/FSH[5]	X										
Cotinine Test, Urine Drug Screen & Alcohol Screen[6]	X	X									
HIV, Hep B, Hep C	X										
Administration of standard meal at dinner		X	X					X			
Administration of mixed meal at breakfast[7]			X	X		X			X		

	Screening Period and Baseline Assessments (within 21 days)			**Treatment Period**							**Follow Up Period or Early Termination**
	Screening	**Day -2**	**Day -1**	**Day 1**	**Days 2-6**	**Day 7**	**Day 8-12**	**Day 13**	**Day 14**	**Day 15**	**Day 22-25**
Pharmacodynamic testing[8]			X	X					X		
Dosing[9]				X	X	X	X	X	X		
Plasma PK Sampling[10]				X	X	X	X		X	X	
Plasma Metabolite Sampling[11]				X					X		
Urinary PK &Metabolite Sampling[12]				X					X		
Bristol Stool Form Scale (BSFS)[13]		←==========→									
Prior and Concomitant Medication	←==========→										
AE Assessment				←==========→							
SAE Assessment	←==========→										
Outpatient Visits	X										X
Inpatient Check-in		X									
Inpatient Check-out										X	

1. Day - 2: Confirm eligibility, update medical / medication history. Ht at Screening only. Wt at screening and on Days -1, 7 and 15. Brief symptom directed physical exam on Days 7, 15 and at FU.
2. Full vital signs will be taken at screening, D-2, D-1, Day 1, 7 and 14 predose, D15, & FU. Vital signs on Day 1, 7 and 14 will be taken 4 hrs after admin of study drug.
3. 12 lead ECG will be on Days 1, 7 and 14 predose and approximately 4 hours after administration of study drug.
4. Serum hCG at screening and follow up, urine hCG pre-dose on Day -2 – all females.
5. If indicated
6. An alcohol breath test is acceptable.
7. Mixed meal breakfast to be eaten on days -1, 1, 7 and 14 immediately following dose (approx 0800) and eaten within 15 minutes.

8. **Blood samples for PD on Day -1, Day 1 and Day 14** at the following timepoints: 15, 10, and 5 minutes prior to breakfast and 0.5, 1, 2, and 4 hrs post breakfast. Lunch to be eaten after 4 hr sample obtained
9. **QD Dosing** will be at approximately 0800 hours and **BID dosing** will be at approximately 0800 and PM dose to be administered 10 hours following AM dose.
10. **For QD dosing**: **Days 1 and 14** PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 0.5,1, 1.5, 2, 2.5, 3, 4, 5, 8, 12, 16, and 24 hr (within 10 minutes or less prior to next dose).
    For QD dosing, **Day 7**: PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 1, 2, 4, 8, and 24 hours (10 minutes prior to next dose on Day 8).
    For BID dosing: **Days 1 and 14** PK blood samples to be obtained predose within 10 minutes prior to 1st dose and then postdose at the following timepoints: 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 8, 10 (10 mins prior to 2nd dose), 11, 12, 14, 16, and 24 hrs (within 10 minutes or less prior to next dose).
    For BID dosing, Day 7: PK blood samples will be obtained predose within 10 minutes prior to 1st dose at time zero and then postdose at the following timepoints: 1, 2, 4, 8, 10 (10 mins prior to 2nd dose) and 24 hrs (10 minutes or less prior to next dose).
11. Plasma samples for metabolite profiling will be collected pre-dose and at all PK timepoints post-dose (for 0-24 hours for all cohorts, however only samples from the highest dose cohort will be analyzed.
12. Urine samples to aid metabolite structure identification will be collected pre-dose and over 0-24 hours for all cohorts and analyzed for the highest dose cohort samples only.
13. BSFS for all stool samples beginning with time of check into clinical facility.

3. INTRODUCTION

Irritable bowel syndrome (IBS) is a relatively common gastrointestinal (GI) illness with a prevalence of approximately 5 to 20% and characterized by a constellation of clinical symptoms including abdominal pain and discomfort, abnormal bowel habits, and bloating [Camilleri, 2012] [Halland, 2013] [Longstreth, 2006]. Because the etiology of the disease has not been established, diagnosis is difficult and relies primarily on the presence of a specific symptom complex occurring with a minimum frequency in the absence of an alternative explanation. It is generally believed that the sensory inputs/outputs in the peripheral and central nervous system are altered in such a way that a patient with IBS has a heightened and disproportionate sensory experience for a given stimulus, i.e. visceral hypersensitivity [Azpiroz, 2007]. Visceral hypersensitivity may result from increased nerve fiber density and sprouting in the intestinal mucosal tissues of patients with IBS [Dothel, 2015].

The only drugs which are FDA-approved for treatment of IBS are alosetron, rifaximin and eluxadoline for refractory diarrhea-predominant IBS, and linaclotide and lubiprostone for constipation-predominant IBS. The majority of these drugs and those used off label to treat IBS, treat patient symptoms by altering GI motility and are minimally effective in addressing abdominal pain and discomfort. Of the approved agents, only alosetron is hypothesized to modulate the central and enteric nervous system. [Berman, 2002] [Gyermek, 1996]

3.1. Study Rationale

The current study is designed to assess the safety, tolerability and PK of escalating single and repeat oral doses of GSK3352589 in normal healthy volunteers. This study is the first administration of GSK3352589 to humans.

3.2. Background

REarranged during Transfection (RET) is a neuronal growth factor receptor tyrosine kinase whose activity is critical for the development of the enteric nervous system, the kidney and spermatogenesis [Airaksinen, 2002]. The role of RET kinase in the development of the ENS has been aided by the study of patients with Hirschsprung's disease who frequently suffer from colonic obstruction due to a lack of normal colonic innervation and possess RET loss of function mutations [Edery, 1994]. While its role during the development of the ENS has been well established, recent reports also implicate a significant role for RET kinase in the maintenance and plasticity of the adult ENS. Indeed, neurons within the submucosal and myenteric plexus of the adult human colon have been shown to express RET kinase and its co-receptors glial cell line-derived neurotrophic factor family receptors alpha 1 and 2 (GFRα1 and GFRα2) while glial cell line-derived neurotrophic factor (GDNF), the ligand for RET kinase, is expressed in the muscularis mucosa, and circular and longitudinal muscle tissue [Barrenschee, 2013]. Systemic administration of GDNF in adult rodents results in significant increases in submucosal neuron density in both the small intestine and colon and altered function [Wang, 2010]. Furthermore, a conditional knockout of the RET kinase co-receptor, GFRα3, results in decreased colonic hypersensitivity implicating a role for RET kinase

signaling in visceral nociception [Tanaka, 2011]. Therefore, by reducing RET signaling, RET kinase inhibition may modulate sensory neuron activity in the enteric nervous system.

GSK3352589 is a potent and selective inhibitor of RET kinase which has been shown to reduce visceral hypersensitivity in an animal model of IBS and inhibit cholinergic-induced increases in colonic motility. The results from these preclinical studies suggest that RET kinase inhibition has the potential to reduce abdominal pain and discomfort in patients with IBS via a novel mechanism of action which may normalize both visceral hypersensitivity and abnormal motility by inhibiting the function of cholinergic neurons. Thus, the patient population that may derive the greatest benefit would consist of individuals with IBS-diarrhea with increased GI motility (IBS-D).

GSK3352589 is the second RET kinase inhibitor to be assessed in normal volunteers. To date, the first RET kinase inhibitor, GSK3179106, has been administered to 48 healthy volunteers in two Phase I clinical studies. In general, administration of GSK3179106 to normal volunteers has been well tolerated. Overall, systemic exposures GSK3179106 were low due to poor oral bioavailability. A significant food effect was observed; the geometric mean ratio comparing fed to fasted for $AUC_{0\text{-}t}$ and C_{max} was approximately 10-fold and 8-fold, respectively. No clinically relevant risks that would preclude dosing a RET kinase inhibitor for up to 2 weeks in normal healthy volunteers have been identified. GSK3352589 has different physicochemical properties than GSK3179106. GSK3352589 is a Biopharmaceutical Classification System (BCS) Class 3 compound with high solubility and low permeability and is expected to have more homogeneous GI tract tissue distribution which has the potential for improved effectiveness. Additionally, GSK3352589 systemic exposures are expected to be lower when compared to GSK3179106 and are not expected to increase when administered with food.

3.2.1. Nonclinical

GSK3352589 is a potent ATP non-competitive reversible inhibitor of the RET kinase enzyme in biochemical and cell-based assays with a mean IC_{50}'s of 0.07 and 21 nM respectively. *In vivo*, the effect of GSK3352589 was assessed in a rat model of irritable bowel syndrome (IBS) for the inhibition of acetic acid induced colonic hypersensitivity as measured by the visceromotor response to colonic distension at different pressures [Plourde, 1997]. GSK3352589 significantly inhibited rat colonic hypersensitivity when administered orally twice daily at 10 mg/kg/dose for 3.5 days when compared to vehicle controls (49 and 43% inhibition at 40 and 60 mmHg, respectively; $P<0.0001$). Similarly, doses of GSK3352589 at 30 and 100 mg/kg/dose inhibited colonic hypersensitivity compared to vehicle controls (30 mg/kg/dose: 43 and 40% inhibition; 100 mg/kg/dose: 45 and 44% inhibition at 40 and 60 mmHg, respectively, $P<0.0001$). GSK3352589 at all doses examined had no effect on stool consistency or colonic motility in the basal state. In contrast, GSK3352589 was able to inhibit increased colonic motility stimulated with the use of the cholinergic agonist neostigmine at doses $\geq$ 3mg/kg/dose.

In addition to its expression in the enteric nervous system, RET kinase is expressed in enteroendocrine cells lining the intestinal mucosa (data on file). Preliminary test results evaluating the effect of a tool compound that inhibits RET kinase suggest a potential role

of RET kinase in regulating levels of postprandial glucagon-like peptide-1 (GLP-1) (data on file). Safety pharmacology studies have shown that GSK3352589 caused no overt treatment-related acute neurobehavioral effects in the conscious rat following single doses of 10, 100 or 1000 mg/kg. GSK3352589 produced no treatment-related effects on the cardiovascular system in conscious dogs. GSK3352589 had no effect on respiratory ventilation parameters in dogs after single doses of 10, 100 or 1000 mg/kg. GSK3352589 produced a concentration-dependent prolongation in the QT interval in rabbit ventricular wedge preparations. Based on this finding, an investigative cardiovascular study was performed in conscious dogs. Intravenous infusions of GSK3352589 yielding plasma concentrations greater than target concentrations determined by the rabbit ventricular wedge assay indicated no ECG waveform abnormalities/ arrhythmias or QT prolongation attributable to GSK3352589. IC_{50} values for inhibition of hERG channel tail current could not be achieved at concentrations up to 44.67 μM, however concentration-dependent inhibition allowed calculation of an IC_{25} which was estimated to be 28.22 μM (15.55 μg/mL). As these *in vitro* concentrations, which represent free drug, greatly exceed those anticipated in humans, it is considered unlikely that GSK3352589 would prolong QT interval at the proposed clinical dose. In addition, as described above, no effects on the ECG measurements, including QT interval measurements, made at regular intervals throughout studies in which animals received GSK3352589 at doses up to 1000 mg/kg/day for 28 days were observed. It is therefore concluded that GSK3352589 is unlikely to cause any effects on the ECG in humans.

No test article-related findings were noted in the 1 month rat and dog oral toxicity studies, therefore the NOAEL was considered to be greater than or equal to 1000 mg/kg/day, the highest dose tested. In dogs, the highest exposure was observed where gender averaged $AUC_{(0-t)}$ and Cmax were 40.4 ng.h/mL and 26.6 ng/mL, respectively; in rats gender averaged AUC(0-t) and Cmax were 51 ng.h/mL and 7.7 ng/mL, respectively.

In a non-GLP study conducted in rats, a dose range of GSK3352589 was given by intravenous infusion, 4 hours daily, for 7 consecutive days to identify target organs at systemic concentrations exceeding predicted clinical exposure. Test article-related effects were limited to cortical lymphocytolysis in the thymus and decreased lymphocyte and total white blood cell counts. Systemic exposure in rats given 5 mg/kg/day by 4 hour daily intravenous infusion produced an AUC(0-t) and Cmax of 2490 ng.h/mL and 578 ng/mL, respectively.

The genetic toxicity of GSK3352589 has been investigated in preliminary Ames and Mouse Lymphoma Assay (MLA) assays. No mutagenic potential was demonstrated in either the absence or presence of S9-mix in these assay systems.

Low systemic exposure following single and repeat dose administration of the oral formulations has been demonstrated in these nonclinical studies, consistent with very low oral bioavailability (rats≤2%; dogs≤1%).

Preliminary data from two non-GLP dose ranging embryofetal development (EFD) studies conducted with GSK3352589 in rats and rabbits demonstrated no findings at exposures much higher than those achievable by oral administration (Cmax 623 ng/mL and AUC 5080 ng.h/mL).

Detailed summaries of all completed in vitro and in vivo nonclinical safety pharmacology and toxicity studies are provided in the GSK33352589 Investigator's Brochure [GlaxoSmithKline Document Number 2016N305051_00].

3.3. Benefit/Risk Assessment

Summaries of findings from non-clinical studies conducted with GSK3352589 can be found in the Investigator's Brochure [GlaxoSmithKline Document Number 2016N305051_00]. The following section outlines the risk assessment and mitigation strategy for this protocol.

3.3.1. Risk Assessment

The current study 207440 represents the first administration of GSK3352589 to healthy subjects and extensive safety monitoring will occur during the course of the study. Considerations for safety monitoring are derived primarily from experience dosing the first RET kinase inhibitor (GSK3179106) to normal healthy volunteers and GSK3352589 non-clinical data. Administration of GSK3179106 in single doses up to 800 mg QD in the fasted state and repeat doses up to 200 mg BID, in the fed state, was in general, well tolerated with no clinically important AEs observed that would preclude dosing of GSK3352589 to normal healthy volunteers for up to 14 days. The safety and tolerability profile of GSK3179106 is attributed to its low oral bioavailability.

Based on the absence of test article related adverse findings in the GLP safety pharmacology and repeat dose toxicity studies, coupled with the likelihood of very low GSK3352589 systemic exposures highest planned AUC exposure = 40.4 ng.h/mL, the risk of adverse events occurring in healthy adult male volunteers and female volunteers of non childbearing potential administered single or repeat doses of GSK3352589 in the fasted or fed state is judged to be low.

More detailed information about the known and expected benefits and risks and reasonably expected adverse events of GSK3352589 may be found in the the Investigator's Brochure [GlaxoSmithKline Document Number 2016N305051_00].

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
Investigational Product (IP) [GSK 3352589]		
Embryofetal development toxicity	Potential class effect of tyrosine kinase inhibitors. Lead RET kinase inhibitor is a multi-species teratogen. Early studies with GSK3352589 suggest that it is not teratogenic. Definitive studies are planned.	Women of child bearing potential excluded from study participation. Men with female partners required to use barrier contraception and females required to use a GSK approved method of hormonal contraception (see

Potential Risk of Clinical Significance	Summary of Data/Rationale for Risk	Mitigation Strategy
		Section 12.7).
Drug drug interaction	Given possibility of CYP3A4 and p-glycoprotein inhibition in gut	Appropriate inclusion/exclusion criteria.
Potential GI effects	Based on biological properties (putative)	Routine PV and BSFS to monitor changes in defecation pattern.

3.3.2. Benefit Assessment

Participants in the study are not expected to receive any direct medical benefits from their participation in the study.

3.3.3. Overall Benefit:Risk Conclusion

Taking into account the measures taken to minimize risk to subjects participating in this study, the potential risks identified in association with GSK3352589 are justified by the eventual benefits that may be afforded to patients with IBS.

4. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary Part A Single Dose • To evaluate the safety and tolerability of single escalating oral doses of GSK3352589 administered in the fasted state and a single dose of GSK3352589 administered in the fed state in healthy adult subjects • To evaluate the pharmacokinetic parameters of escalating single oral doses of GSK3352589 under fasting and fed (for one dose only) conditions in healthy adult subjects. Part B Repeat Dose • To evaluate the safety and tolerability of repeat escalating oral doses of GSK3352589 administered once or twice daily in the fasted or fed state. • To characterize the PK of GSK3352589 of repeat escalating oral doses administered once or twice daily in the fasted or fed state	• Clinical safety and tolerability data including spontaneous adverse event (AE) reporting, clinical observations, physical examination findings, 12-lead electrocardiograms (ECG), vital signs, Bristol Stool Form Scores (BSFS), clinical laboratory tests. • PK parameters obtained following administration of GSK3353589. PK parameters include areas under the plasma concentration versus time curve, AUC over the dosing interval and other time intervals, Cmax, Tmax, and terminal t ½, for Days 1 and 14 will be analyzed as data permit.
Exploratory • To explore the effect of GSK3352589 on the postprandial plasma profiles of total glucagon like peptide-1 (GLP-1) and total peptide YY (PYY) following repeat oral doses of GSK3352589 administered once or twice daily • To investigate the biotransformation of GSK3352589 in plasma and urine	• Postprandial challenge (mixed-meal) profiles of total GLP-1 and total PYY. • Provide samples of plasma and urine for the identification of any compound-derived metabolite(s).

5. STUDY DESIGN

5.1. Overall Design

This randomized, double-blind (sponsor unblinded), placebo controlled, dose escalation study will be divided into two parts: Part A is a single dose escalating, four period

crossover (Cohort 1) with a two period, single sequence pilot food effect (Cohort 2) study and Part B is a 14 day repeat dose escalating, ascending cohort study.

The study will be composed of 3 periods for all subjects (Screening, Treatment, and Follow-up). Subjects will participate in either Part A or Part B. A subject's total time involved in the study will be approximately 10 weeks for subjects enrolled in Part A, Cohort 1 and approximately 8 weeks for subjects enrolled in Part A, Cohort 2. Subjects enrolled in Part B will be involved in the study for a total time of approximately 7 weeks.

All dosing periods for subjects participating in Part A will be completed before beginning enrollment in Part B.

5.2. Number of Participants

5.2.1. Part A

Single dose, dose escalating, four period, crossover (Cohort 1) with a two period, single sequence pilot food effect (Cohort 2) study.

A sufficient number of healthy volunteers will be screened to enroll 16 subjects (8 subjects/cohort). Two cohorts of subjects are planned. Subjects assigned to Cohort 1 will participate in 4 dosing periods: 1 placebo and 3 dose escalating periods. The planned dose range for Cohort 1 is 2 to 400 mg. Each dosing period during Cohort 1 will be staggered so that only 2 of the 8 subjects will be administered study drug initially. Once 24 hours have elapsed, and provided there are no safety concerns, the remainder of subjects scheduled for that dosing period may be dosed. A review of safety and tolerability will occur prior to administration of the next dose level. This same procedure will be followed for each escalating dosing period. Within each cohort, subjects will return for their next scheduled dosing period approximately 14 days after administration of the study drug during the prior dosing period.

Cohort 2 will proceed after completion of the treatment periods in Cohort 1. Subjects assigned to Cohort 2 will participate in a pilot food effect in Periods 1 and 2 (see Table 1 below). A single dose of GSK3352589 will be administered in the fasted and fed states in Cohort 2. The dose selected to evaluate the food effect will allow for a 2X increase in bioavailability and predicted exposures will not exceed the highest observed exposures at prior doses.

The actual doses to be administered may be adjusted based on safety, tolerability and PK data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose for Cohort 1. Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits. Preliminary safety and PK data from all subjects will be reviewed prior to the next Dosing Period. The decision to proceed to the next dose level will be made by the GSK Medical Monitor and the investigator based on safety, tolerability and available PK data.

Table 1 Part A Treatment Sequence

Cohort 1 (Single Dose Escalation[1])

Treatment Sequence	Subjects	Period 1	Period 2	Period 3	Period 4
A	PPD and PPD	Placebo	D2	D3	D4
B	and	D1	Placebo	D3	D4
C	and	D1	D2	Placebo	D4
D	and	D1	D2	D3	Placebo

1 Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits.

Cohort 2 (Pilot Food Effect)

Treatment Sequence	Subjects	Period 1	Period 2
E	PPD	D5	D5 + food
F	PPD and PPD	Placebo	Placebo + food

5.2.2. Part B

Part B is a 14 day randomized, double-blind, placebo-controlled, repeat dose escalating, ascending cohort study.

Up to six (6) cohorts are planned and will be conducted sequentially. A sufficient number of healthy volunteers will be screened to randomize 48 subjects (8 subjects/ cohort). If subjects are prematurely discontinued from the study, additional replacement subjects may be enrolled at the discretion of the Sponsor in consultation with the Investigator.

The planned dose range is 2 mg once daily to 200 mg twice daily. The starting dose for Part B will be selected after review of the safety, tolerability and PK data of subjects enrolled in Part A and completing all dosing periods. An example dose escalation plan is shown in Table 2 below. The actual doses to be administered may be adjusted based on safety, tolerability and PK data at previous dose levels; these dose adjustments may involve either an increase or a decrease in the planned dose. Dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits.

Table 2 Planned Dose Escalation for Part B

	Regimen Descriptions	Number of Subjects
Cohort 1	2 mg of GSK3352589 administered QD or matching placebo administered QD	GSK3352589 n=6 Placebo n=2
Cohort 2	10 mg of GSK3352589 administered QD or matching placebo administered QD	GSK3352589 n=6 Placebo n=2
Cohort 3	25 mg of GSK3352589 administered BID or matching placebo administered BID	GSK3352589 n=6 Placebo n=2

	Regimen Descriptions	Number of Subjects
Cohort 4	50 mg of GSK3352589 administered BID or matching placebo administered BID	GSK3352589 n=6 Placebo n=2
Cohort 5	100 mg of GSK3352589 administered BID or matching placebo administered BID	GSK3352589 n=6 Placebo n=2
Cohort 6	200 mg of GSK3352589 administered BID Or matching placebo administered BID	GSK3352589 n=6 Placebo n=2

Subjects will undergo pretreatment baseline pharmacodynamic (PD) testing after administration of a breakfast mixed-meal challenge on Day -1 prior to receiving the first dose of study drug, on Day 1, and at the end of the treatment period on Day 14.

Preliminary safety and tolerability from 14 days of dosing and PK data from 7 days of dosing for each cohort will be reviewed prior to dose escalation and will be used to determine the dose to be administered in the subsequent cohort. The decision to proceed to the next dose level will be made by the GSK Medical Monitor and the investigator based on safety, tolerability and available PK data.

Additional subjects/cohorts may be enrolled to allow for evaluation of additional dose levels or dosing regimens.

5.3. Participant and Study Completion

A participant is considered to have completed the study if he/she has completed all phases of the study including the follow up visit

The end of the study is defined as the date of the last visit of the last participant in the study.

5.4. Scientific Rationale for Study Design

5.4.1. Part A

A randomized, placebo-controlled, double-blind (sponsor unblind), single ascending dose crossover study design is based on well established and published methods to evaluate the single dose administration of experimental drugs. The use of this design reduces inter-subject variability in the PK data, thus improving its quality and interpretation. Additionally, it reduces the number of subjects exposed to the investigational agent and required to guide selection of the starting dose for the repeat dose study to follow. Approximately a 2 week washout period between administrations of escalating doses assures that study drug has been cleared and in the event that an adverse event occurs, adequate time for resolution. Placebo has been selected as the control.

A pilot food effect study is included because of the potential for a food effect with a high solubility, low permeability compound such as GSK3352589. Theoretical potential exists for a decrease in bioavailability that could limit tissue exposure.

Placebo has been selected as the control.

5.4.2. Part B

A randomized, placebo-controlled, double-blind (sponsor unblind), ascending cohort study design is based on well established and published methods to evaluate the repeat dose administration of experimental drugs. Placebo has been selected as the control.

5.5. Dose Justification

In the preclinical safety and repeat dose toxicity studies performed to date, GSK3352589 1000 mg/kg administered for up to 28 days by the oral route of administration, results in low exposure in rats or dogs without evidence of a test article related safety signal or finding. Thus, the starting dose of 2 mg is derived from the minimum anticipated biological effect level (MABEL) dose related to its pharmacology rather than to NOAELs in the absence of safety or toxicity findings in animals [CDER, 2005].

5.5.1. Human Pharmacodynamic Predictions

Five methods were used to estimate a therapeutic dose range for GSK3352589. The target tissue is the ENS within the colon. The pharmacological activity in the rat IBS visceral hypersensitivity model is driven by drug penetration from colon luminal contents to the ENS within colon tissue. The pharmacological response correlates with colon tissue concentrations rather than plasma concentrations.

Because the pharmacology is thought to be driven by colon tissue drug concentrations, the human dose predictions are independent of the predicted human systemic pharmacokinetic profile. A summary of the results for the various methods for translating rat to human pharmacology and doses are shown in Table 3. The dose range for minimal pharmacological effect is predicted to be 5 mg to 136 mg once daily. The dose range for maximum pharmacological effect is predicted to be 5 mg to 454 mg once daily. It is unknown if these doses are best given once or twice daily, but twice daily dosing should provide more sustained tissue colon concentrations per day. The rat IBS visceral hypersensitivity model indicates steady state tissue concentrations and twice daily dosing is required. Therefore low, single doses in humans are not expected to have an effect. Detailed explanations of the methods are shown in Section 12.2.

Table 3 Summary of human dose predictions by method

Method	***Pharmacologically Active Doses***	
	Minimum Effect	**Maximum Effect**
Rat IBS model dose reference	3 mg/kg	10 mg/kg
Systems pharmacology model	5-10 mg QD or >2 mg BID	
HED - body surface area extrapolation	~30 mg	~100 mg
HED with relative small intestine surface area correction (rat to human)	~7 mg	~23 mg
HED with relative large intestine surface area correction (rat to human)	~136 mg	~454 mg
Human dose scaled by colon content and gut weight	~122 mg	~406 mg

HED = human equivalent dose (FDA- CDER 2005)

5.5.2. Human Pharmacokinetic Predictions

The model PK parameters that relate dose and plasma GSK3352589 concentrations were based on several interspecies scaling and prediction methods. Small molecule drug candidates with moderately high hepatic clearance and any size volumes of distribution are more reliably scaled between species based on body weights and a few other methods. Absolute bioavailability and absorption rates are estimated more directly from the animal data. The absolute bioavailability of GSK3353589 is expected to be very low and therefore the primary source of uncertainty for the predictions of concentration-time profiles and exposure estimates (i.e., AUC, Cmax).

The systemic clearance (CLs) was obtained from five interspecies scaling methods. The CLs median (range) was 836 mL/min (528-1002 mL/min). The magnitude of the CLs estimate implies GSK3352589 is a moderately high hepatic clearance compound. The median volume of distribution (V) was 314 liters ranging from 126 to 546 liters and implies drug distribution is very large and extensively distributed into tissues. Consequently, the median (range) for plasma half-life (T1/2) is 3.7 (2.1-7.9) hours. The Tmax, time to reach Cmax, is expected to be about 1.5 hours ranging from 0.5 to 5.0 hours. The variability in all parameters was taken into account with simulations that include probability distributions for each parameter in the PK model equation describing the GSK3352589 concentration time profiles.

In all nonclinical species tested, GSK3352589 has highly variable, limited, and less than proportional systemic exposure with increasing doses, consistent with its very low oral bioavailability (rats ≤2%; dogs ≤1%). In the rat, oral administration with food slightly increased the systemic exposure of GSK3352589. The bioavailability (F) estimates used in the simulations are listed from low to high: 0.016% (mice solution, fasted), 0.02% (male rats GLP TK suspension, fed), 0.06% (rats suspension, fed), 0.07% (female rats GLP TK suspension, fed), 0.18% (rats solution, fasted), 0.24% (dogs solution, fed), 1.1% (rats non GLP TK suspension, fed), 1.9% (rats non GLP TK suspension, fed). The F ranges from 0.016% to 1.9% and attributed to large variation in individual rats. The overall group median is 0.125%. More weight was given to the estimates of F that included all species and the predicted AUC and Cmax exposures for administration of single doses based on most likely bioavailability using F=0.16% can be seen in the Table 4 below. The higher estimates of 1.1% and 1.9% in rats appeared to be the outliers compared to other rat estimates, mice and dogs and the impact of assuming the highest potential exposure (1.5%) is shown in Table 6. These predictions were assumed to be linearly related to dose. This assumption is conservative since animal PK shows either linear or less than dose proportional relationships. Plasma GSK3352589 concentration time profiles are shown in Figure 1 for two single doses (2 mg and 200 mg). The predicted AUC and Cmax exposures for repeat doses at steady state are shown in Table 5.

The bioavailability of GSK3352589B is likely permeability-limited since it has low permeability and high FaSSIF solubility and considered a biopharmaceutical classification system (BCS) Class III type compound. Therefore, the compound is expected to be solubilized and well mixed in the gut contents and absorption across the enterocyte epithelial layer is the rate limiting step. MALDI imaging studies indicate good GI tissue distribution despite a low permeability categorization. With BCS class III compounds, it is more likely that there will be a decrease in bioavailability, where plasma AUC and Cmax are decreased [Radwan, 2012]. However, a food effect study in rats with rat chow showed an increase in plasma AUC of 2.6 fold. The mixed prediction of a food effect will be considered when dose selection is made for the pilot food effect study.

Table 4 Predicted exposures based on mostly likely bioavailability estimates for single doses - Median (5th and 95th percentiles) predictions for plasma GSK3352589 AUC(0-∞) and Cmax (FASTED) by dose

Dose (mg)	AUC(0-∞)[a] (h*ng/mL)	Cmax[a] (ng/mL)
1	0.021 (0.0078, 0.095)	0.0028 (0.0013, 0.011)
2	0.044 (0.015, 0.19)	0.0058 (0.0026, 0.022)
5	0.105 (0.039, 0.48)	0.015 (0.0065, 0.06)
10	0.211 (0.077, 0.95)	0.029 (0.013, 0.11)
25	0.55 (0.19, 2.4)	0.075 (0.033, 0.29)
50	1.09 (0.39, 4.8)	0.15 (0.065, 0.57)
100	2.18 (0.77, 9.5)	0.29 (0.129, 1.14)
200	4.36 (1.53, 19.0)	0.579 (0.258, 2.27)
400	8.72 (3.06, 38.0)	1.16 (0.52, 4.54)

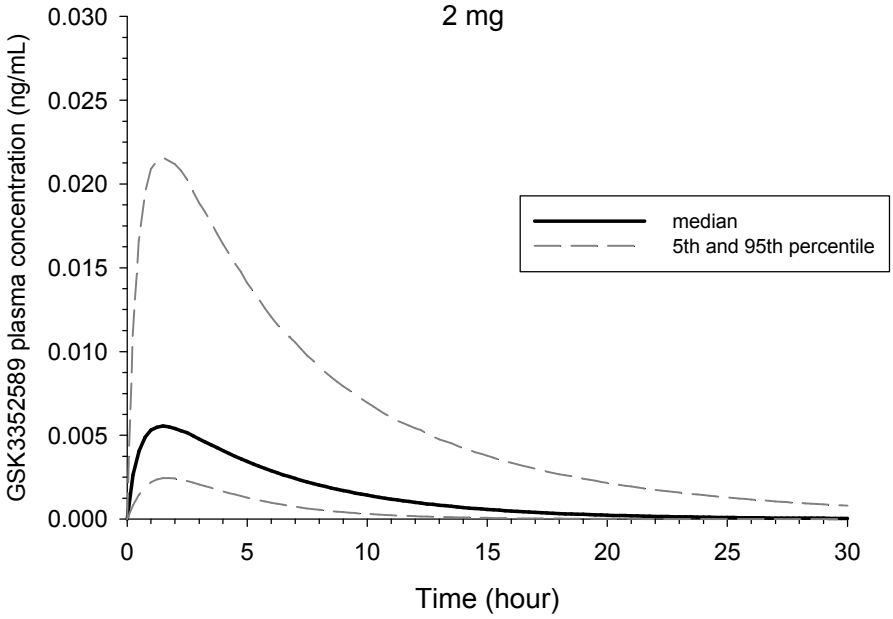
a. median (5th, 95th percentiles), n=10,000 simulations,
Additional Output: median (range) Tmax (h) = 1.5 (0.5-5.0); median [(5th, 95th percentiles) and range] T1/2(h) = 3.7 [(2.1, 7.9) and 1.6-12],
Predictions assume dose proportionality and this assumption is generally consistent with animal PK though with highly variability,
Bioavailability (F) average 0.16% ranging from 0.016%-1.5%; F is built into V/F as input variable, the 5th, 95th percentiles include the low and high F estimates,
Input: median (5th, 95th percentiles) for K01(h^{-1}) = 1.7 (0.66, 3.9); K10 (h^{-1}) = 0.19 (0.09, 0.33); Vd/F (Liters) = 262,858 (67,595- 568,451); mean and range for Vd (Liters) = 314 (126-546),
Single dose human exposures AUC(0-∞) are equivalent to steady state AUC(0-24 h) with once daily dosing.

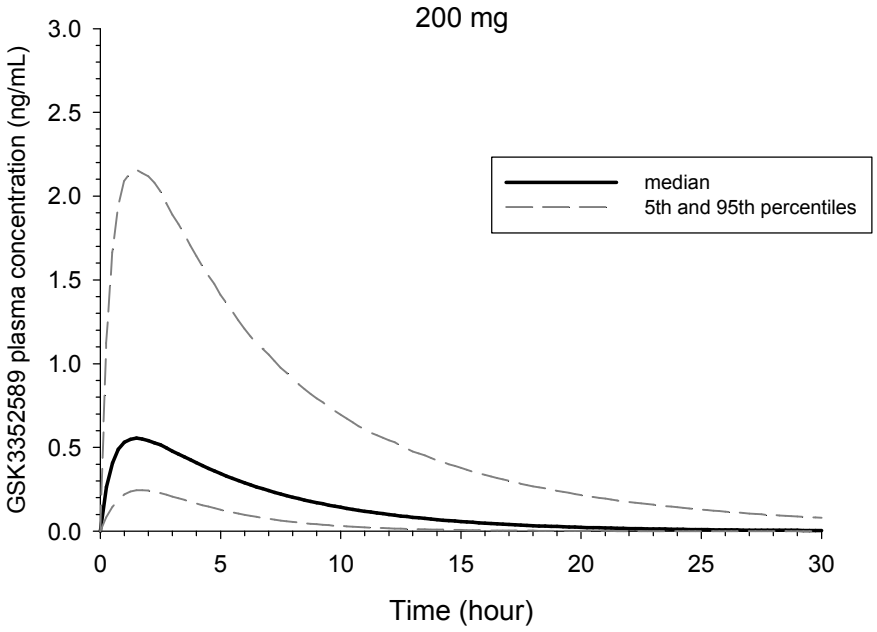
Table 5 Predicted exposures based on mostly likely bioavailability estimates for repeat doses at steady state - Median (5th and 95th percentiles) predictions for plasma GSK3352589 AUCss (FASTED) by dose

	Once Daily Dosing	Twice Daily Dosing
Dose (mg)	**AUCss(0-24) (h*ng/mL) [a]**	**AUCss(0-24) (h*ng/mL) [a]**
1	0.021 (0.0078, 0.095)	0.042 (0.016, 0.19)
2	0.044 (0.015, 0.19)	0.088 (0.030, 0.38)
5	0.105 (0.039, 0.48)	0.210 (0.078, 0.96)
10	0.211 (0.077, 0.95)	0.422 (0.154, 1.90)
25	0.55 (0.19, 2.4)	1.10 (0.38, 4.80)
50	1.09 (0.39, 4.8)	2.18 (0.78, 9.6)
100	2.18 (0.77, 9.5)	4.36 (1.54, 19.0)
200	4.36 (1.53, 19.0)	8.72 (3.1, 38.0)
400	8.72 (3.06, 38.0)	-

a. median (5th, 95th percentiles), n=10,000 simulations.
Predictions assume dose proportionality and this assumption is generally consistent with animal PK though highly variability.
Single dose human exposures AUC(0-∞) are equivalent to steady state AUCss(0-24 h) with once daily dosing assuming no time dependent changes in drug disposition.

Figure 1 Simulation of median (5th, 95th percentiles) concentration-time profiles in humans for 2 mg and 200 mg single doses of GSK3352589 assuming most likely bioavailability





5.5.2.1. Predicted exposures for higher than expected bioavailability

The PK parameter that is most uncertain is oral bioavailability (F). Although exposures remain very low, results of preclinical PK and TK assessments suggest the potential for up to a 100X increase in bioavailability. An analysis of predicted exposures for the highest potential oral bioavailability, 1.5% (gender averaged) is included to illustrate the effect of bioavailability on AUC and Cmax for single doses in Table 6 below. All other parameters predictions are the same as in Table 5 above.

Table 6 Predicted exposures based on highest possible bioavailability estimates for single doses - Median (5th and 95th percentiles) predictions for plasma GSK3352589 AUC(0- ∞) and Cmax (FASTED) by dose

Dose (mg)	AUC(0-∞)[a] (h*ng/mL)	Cmax[a] (ng/mL)
1	0.26 (0.128, 0.61)	0.036 (0.024, 0.06)
2	0.52 (0.26, 1.22)	0.072 (0.048, 0.12)
5	1.30 (0.64, 3.04)	0.18 (0.12, 0.30)
10	2.60 (1.27, 6.07)	0.36 (0.23, 0.59)
25	6.5 (3.2, 15.2)	0.90 (0.60, 1.5)
50	13.0 (6.4, 30.3)	1.8 (1.2, 3.0)
100	26.0 (12.8, 60.5)	3.6 (2.3, 6.0)
200	52.0 (25.5, 121)	7.11 (4.61, 11.9)
400	104 (51, 242)	14.2 (9.2, 24)

a. median (5th, 95th percentiles), n=10,000 simulations,
Additional Output: median (range) Tmax (h) = 1.5 (0.5-5.0); median [(5th, 95th percentiles) and range] T1/2(h) = 3.7 [(2.1, 7.9) and 1.6-12],
Predictions assume dose proportionality and this assumption is generally consistent with animal PK though with highly variability,
Bioavailability (F) = 1.5% for outlier simulations.
Input: median (5th, 95th percentiles) for K01(h^{-1}) = 1.7 (0.66, 3.9); K10 (h^{-1}) = 0.19 (0.09, 0.33); Vd/F (Liters) = 21,353 (13,131- 30332); mean and range for Vd (Liters) = 314 (126-546),
Single dose human exposures AUC(0-∞) are equivalent to steady state AUC(0-24 h) with once daily dosing.

5.5.3. Dose Range and Safety Margins

The MABEL for this therapeutic is related to its pharmacology rather than to any safety or toxicity findings. The lowest doses predicted to be minimally effective following repeat dosing to steady-steady are 5 mg QD or 2 mg BID. The proposed starting dose for this single dose study is 2 mg, a conservative estimate for minimal, if any, effect. The highest doses predicted to be maximally effective following repeat dosing to steady state are 406 – 454 once daily. A highest total daily dose of 400 mg was selected based on the predictions for the maximally effective dose.

Table 7 provides the predicted systemic exposures for administration of single doses up to 400 mg of GSK3352589, assuming linearity for both the most likely estimate for bioavailability (0.16%) and the highest expected estimate for bioavailability (1.5%), and the estimated safety margins for these predicted exposures, respectively. The safety

margins are calculated from the systemic exposures observed in the 4 wk oral dog repeat dose toxicity study. No test article-related findings were noted in the 1 month rat and dog oral toxicity studies, therefore the NOAEL was considered to be greater than or equal to 1000 mg/kg/day, the highest dose tested in each species. In rats, the highest gender-averaged exposures observed were AUC(0-t) and Cmax 51.0 ng.h/mL and 7.7 ng/mL, respectively; and in dogs the gender-averaged AUC(0-t)) and Cmax were 40.4 ng.h/mL and 26.7 ng/mL, respectively. Although these exposures are similar, the dog exposures were used to calculate the safety margins because of the higher Cmax.

The safety margins for Cmax and AUC for the planned starting single dose of 2 mg GSK3352589 assuming the highest possible bioavailability (1.5%) are 742X and 155X, respectively. The predicted mean steady-state Cmax and AUC for humans assuming dose proportionality administered the highest planned single dose of 400 mg are below the animal safety exposure limits if the exposures suggest that bioavailability is closer to the most likely predicted value, 0.16%. During both Part A (single dose) and Part B (repeat dose), dose escalation will occur only if mean systemic exposures are projected not to exceed the defined plasma toxicokinetic (TK) limits [AUC(0-t)) and Cmax of 40.4 ng.h/mL and 26.7 ng/mL, respectively]. The risk of either exceeding the defined TK limits or occurrence of systemic adverse events is judged to be low because of the low oral bioavailability of GSK3352589.

Table 7 Predicted Most Likely and Highest Potential Human Systemic Exposures and Estimated Safety Margins for Single Doses of GSK3352589 (Fasted State)

	Predicted Human Mean Cmax ng/mL		Predicted Human Mean AUC ng.h/mL		4 Week Oral Dog Repeat Dose Toxicity Study*	
	Most Likely	Highest Potential	Most Likely	Highest Potential	Safety Margins for Cmax	Safety Margins for AUC
2 mg	0.006	0.036	0.04	0.26	742 to > 9500X	155-1923X
10 mg	0.029	0.36	0.211	2.6	74 – 920X	16-192X
50 mg	0.15	1.8	1.09	13	15-178X	3.1-37X
200 mg	0.579	7.11	4.36	52	3.8-46X	0.8-9.3X
400 mg	1.16	14.2	8.72	104	1.9-23X	0.4-4.6X

*Safety margins are calculated with the dog NOAELs from the 1 month repeat dose toxicity study because mean Cmax was greater (26.7 ng/mL) than in rats (7.7 ng/mL) and the AUCs were similar (40.4 and 51 ng.h/mL for dogs and rats, respectively).

Although a negative food effect is predicted for this BCS III compound, the dose selected to evaluate the food effect will allow for a 2X increase in bioavailability due to the slight increase in bioavailability observed in the rat food effect study.

6. STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

6.1. Inclusion Criteria

Participants are eligible to be included in the study only if all of the following criteria apply:

AGE
1 Between 18 and 55 years of age inclusive, at the time of signing the informed consent.
TYPE OF SUBJECT AND DIAGNOSIS INCLUDING DISEASE SEVERITY
2 Healthy as determined by the investigator based on a medical evaluation including medical history, physical examination, laboratory tests and cardiac monitoring. A subject with a clinical abnormality or laboratory parameter(s) which is/are not specifically listed in the inclusion or exclusion criteria, outside the reference range for the population being studied may be included only if the investigator, in consultation with the Medical Monitor, agree and document that the finding is unlikely to introduce additional risk factors and will not interfere with the study procedures. 3 History of regular bowel habits and no more than 3 bowel movements per day
SEX
4 Male or Female **a. Male participants:** A male participant must agree to use contraception as detailed in Section 12.7 of this protocol during the treatment period and for at least 7 days, corresponding to time needed to eliminate study treatment for both genotoxic and teratogenic study treatments after the last dose of study treatment and refrain from donating sperm during this period. **b. Female participants:** A female participant is eligible to participate if she is not a woman of childbearing potential (WOCBP) as defined in Section 12.7.
INFORMED CONSENT
5 Capable of giving signed informed consent as described in Section 12.5 which includes compliance with the requirements and restrictions listed in the informed consent form (ICF) and in this protocol.

6.2. Exclusion Criteria

Participants are excluded from the study if any of the following criteria apply:

CONCURRENT CONDITIONS/MEDICAL HISTORY (INCLUDES LIVER FUNCTION AND QTc INTERVAL)
1. ALT and bilirubin >1.5xULN (isolated bilirubin >1.5xULN is acceptable if bilirubin is fractionated and direct bilirubin <35%).
2. Previous Diagnosis of IBS
3. Estimated Glomerular Filtration Rate < 60 mL/min/1.73m^2
4. Current or chronic history of liver disease, or known hepatic or biliary abnormalities (with the exception of Gilbert's syndrome or asymptomatic gallstones)
5. History of GERD, dyspepsia, GI bleeding, diverticulitis, diverticular stricture or other intestinal strictures, GI surgery that could affect motility, bezoars, dysphagia to solid food or pills, pelvic surgery within the past 3 months.
6. Cardiac pacemakers or other implanted electromedical devices.
7. Presence of hepatitis B surface antigen (HBsAg) at screening or within 3 months prior to first dose of study treatment
8. Positive hepatitis C antibody test result at screening or within 3 months prior to first dose of study treatment. NOTE: Participants with positive Hepatitis C antibody due to prior resolved disease can be enrolled, only if a confirmatory negative Hepatitis C RNA test is obtained
9. A positive pre-study drug/alcohol screen.
10. A positive test for HIV antibody.
11. History of regular alcohol consumption within 6 months of the study defined as: • an average weekly intake of >14 standard drinks. One standard drink is equivalent to 10 g of alcohol: 285 ml of beer, 100 ml of wine or 30 ml of 40% alcohol by volume distilled spirits.
12. Current smoker or use of nicotine containing products within the past 3 months or unable to abstain from smoking tobacco or the use of nicotine-containing products while on study or positive pre-study cotinine test.
13. Unable to refrain from consumption of red wine, seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug until inpatient check-out from the clinic.
14. QTcF>450 msec NOTES: • The QTc is the QT interval corrected for heart rate according to Bazett's formula (QTcB), Fridericia's formula (QTcF), and/or another method, machine-read or manually over-read.

• The specific formula that will be used to determine eligibility and discontinuation for an individual subject should be determined prior to initiation of the study. In other words, several different formulae cannot be used to calculate the QTc for an individual subject and then the lowest QTc value used to include or discontinue the subject from the trial. • For purposes of data analysis, QTcB, QTcF, another QT correction formula, or a composite of available values of QTc will be used as specified in the Reporting and Analysis Plan (RAP).
CONCOMITANT MEDICATIONS
15. History of sensitivity to any of the study medications, or components thereof or a history of drug or other allergy that, in the opinion of the investigator or GSK Medical Monitor, contraindicates their participation. 16. Unable to refrain from the use of prescription or non-prescription drugs, including vitamins, antacids, herbal and dietary supplements (including St John's Wort) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) prior to the first dose of study medication for each dosing, unless in the opinion of the Investigator and GSK Medical Monitor the medication will not interfere with the study procedures or compromise subject safety. Paracetamol (≤ 2 grams per day) is acceptable.
RELEVANT HABITS
17. The subject has participated in a clinical trial and has received an investigational product within the following time period prior to the first dosing day in the current study: 30 days, 5 half-lives or twice the duration of the biological effect of the investigational product (whichever is longer). 18. Exposure to more than 4 investigational medicinal products within 12 months prior to the first dosing day 19. Unwillingness or inability to follow the procedures outlined in the protocol. 20. Where participation in the study would result in donation of blood or blood products in excess of 500 mL within a 56 day period.

6.3. Lifestyle Restrictions

6.3.1. Part A Fasted: Meals and Dietary Restrictions

- Subjects must fast from all food and drink (except water) for at least 8 hours prior to dose and PK sampling on Day 1 and will remain fasted for approximately 4 hours postdose. Water may be consumed ad libitum.
- Subjects will consume a standard weight neutral-meal for all meals and with no time limits.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed

within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.

- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.2. Part A Fed for Pilot Food Effect (Cohort 2, Dosing Period 2): Meals and Dietary Restrictions

- Subjects must fast from all food and drink (except water) for at least 8 hours prior to dose and PK sample on Day 1. Water may be consumed ad libitum.
- Subjects will consume a mixed meal breakfast (also referred to as the PD meal in Part B instructions) immediately following the dose on Day 1. This identical mixed meal breakfast will consist of 55% carbohydrate, 15% protein, and 30% fat, representing 20% of subject's total calorie intake**.**
- The mixed meal breakfast must be eaten within 15 minutes and eaten in total.
- Subjects will consume a standard weight-neutral meal for all other meals and with no time limits.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.
- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.3. Part B QD Fasted: Meals and Dietary Restrictions

- On PK days (Day 1, Day 7 and Day 14) Subjects must fast from all food and drink (except water) for at least 8 hours prior to dose and PK sample. Subjects will remain fasted until mixed meal (PD) breakfast meal at approximately 2 hours postdose. Water may be consumed ad libitum.
- On PD days (Day -1, Day 1 and Day 14) subjects must fast from all food and drink (except water) for at least 8 hours prior to PD sample. Subjects will remain fasted until breakfast at approximately 2 hours postdose. Water may be consumed ad libitum.
- On the PD days (Day -1, Day 1, and Day 14) at approximately 2 hours postdose, an identical mixed meal breakfast consisting of 55% carbohydrate, 15% protein, and 30% fat, representing 20% of subject's total calorie intake will be consumed**.**
- The PD breakfast meal must be eaten within 15 minutes and eaten in total.

- The evening before PD testing days (Day -2, Day -1 and Day 13), subjects will consume a standard dinner, identical on all days.
- Subjects will consume a standard weight neutral-meal for all other meals on PK and PD days as well as non PK and PD days with no time limits.
- Lunch will be eaten approximately 4 hours after breakfast and dinner approximately 5 hours after lunch.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.
- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.4. Part B BID Fasted: Meals and Dietary Restrictions

- On PK days (Day 1, Day 7 and Day 14) Subjects must fast from all food and drink (except water) for at least 8 hours prior to AM dose and PK sample. Subjects will remain fasted until mixed meal (PD) breakfast meal at approximately 2 hours postdose. Water may be consumed ad libitum.
- On PK days (Day 1, Day 7 and Day 14) subjects must fast from all food and drink (except water) for at least 1 hour after PM dose administration. Water may be consumed ad libitum.
- On PD days (Day -1, Day 1 and Day 14) subjects must fast from all food and drink (except water) for at least 8 hours prior to PD sample. Subjects will remain fasted until breakfast at approximately 2 hours postdose. Water may be consumed ad libitum.
- On PD days (Day -1, Day 1, and Day 14) subjects will receive an identical mixed meal breakfast at 1000 consisting of 55% carbohydrate, 15% protein, and 30% fat, representing 20% of subject's total calorie intake will be consumed.
- The PD breakfast meal must be eaten within 15 minutes and eaten in total.
- The evening before PD testing days (Day -2, Day -1 and Day 13), subjects will consume a standard dinner, identical on all days.
- Lunch will be eaten at approximately 4 hours after breakfast meal and dinner approximately 5 hours after lunch.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.
- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or

fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.5. Part B QD Fed: Meals and Dietary Restrictions

- On PK days (Day 1, Day 7 and Day 14) Subjects must fast from all food and drink (except water) for at least 8 hours prior to dose and PK sample. Subjects will consume a PD breakfast meal immediately following dosing. Water may be consumed ad libitum.
- On PD days (Day -1, Day 1 and Day 14) subjects must fast from all food and drink (except water) for at least 8 hours prior to PD sample. Subjects will consume breakfast immediately following dosing. Water may be consumed ad libitum.
- On the PD days (Day -1, Day 1, and Day 14), an identical mixed meal breakfast consisting of 55% carbohydrate, 15% protein, and 30% fat, representing 20% of subject's total calorie intake will be eaten immediately following administration of study drug**.**
- The PD breakfast meal must be eaten within 15 minutes and eaten in total.
- The evening before PD testing days (Day -2, Day -1 and Day 13), subjects will consume a standard dinner, identical on all days.
- Subjects will consume a standard weight-neutral meal for all other meals on PK and PD days as well as non PK and PD days and with no time limits.
- Lunch will be eaten at approximately 4 hours after breakfast and dinner approximately 6 hours after lunch.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.
- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.6. Part B BID Fed: Meals and Dietary Restrictions

- On PK days (Day 1, Day 7 and Day 14) Subjects must fast from all food and drink (except water) for at least 8 hours prior to dose and PK sample. Subjects will consume the PD breakfast meal immediately following dosing. Water may be consumed ad libitum.
- On PD days (Day -1, Day 1 and Day 14) subjects must fast from all food and drink (except water) for at least 8 hours prior to PD sample. Subjects will consume breakfast immediately following dosing. Water may be consumed ad libitum.

- On the PD days (Day -1, Day 1, and Day 14), an identical mixed meal breakfast consisting of 55% carbohydrate, 15% protein, and 30% fat, representing 20% of subject's total calorie intake will be consumed.
- The PD breakfast meal must be eaten within 15 minutes and eaten in total.
- The evening before PD testing days (Day -2, Day -1 and Day 13), subjects will consume a standard dinner, identical on all days.
- Subjects will consume a standard weight neutral meal for all other meals on PK and PD days as well as non PK and PD days and with no time limits.
- Lunch will be eaten at approximately 4 hours after breakfast and dinner approximately 6 hours after lunch.
- Each dose of study drug will be administered with 240 mL of water while the subject is sitting/ standing. All of the water must be completely consumed within approximately 5 minutes. Subjects may sit upright or stand, but must not lie down for at least 30 minutes after study drug administration.
- Subjects must refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice and/or pummelos, exotic citrus fruits, grapefruit hybrids or fruit juices from 2 days prior to the first dose of Study Drug on Day 1 until inpatient check-out from the clinic.

6.3.7. Caffeine, Alcohol, and Tobacco

- Subjects will abstain from alcohol for 24 hours prior to Day -1 to the clinic until inpatient check-out from the clinic.
- The use of nicotine-containing products (including nicotine patches) will not be permitted from 3 months prior to admission to the clinic until inpatient check-out from the clinic.

6.3.8. Activity

- Participants will abstain from strenuous exercise for 48 hours before each blood collection for clinical laboratory tests. Participants may participate in light recreational activities during studies (eg, watching television, reading).

6.4. Screen Failures

Screen failures are defined as participants who consent to participate in the clinical study but are not subsequently randomized. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse events (SAEs).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened.

7. TREATMENTS

Study treatment is defined as any investigational treatment or placebo intended to be administered to a study participant according to the study protocol.

7.1. Treatments Administered

Study Treatment Name:	GSK3352589	Placebo
Dosage formulation:	Tablet	Tablet
Unit dose strength(s)/Dosage level(s):	1, 5, 25 and 100 mg	Placebos to match actives across all strengths
Route of Administration	Oral	Oral
Dosing instructions:	Swallow whole with water, do not chew	Swallow whole with water, do not chew
Packaging and Labeling	Study Treatment will be labeled as required per country requirement.	Study Treatment will be labeled as required per country requirement.
Manufacturer	WUXI/ GlaxoSmithKline	WUXI/ GlaxoSmithKline

7.2. Dose Modification

This protocol allows some alteration from the currently outlined dosing schedule but the (predicted) maximum/cumulative exposure will not exceed a CMAX=26.6 ng/mL or AUC = 40.4 ng.h/mL. The decision to proceed to the next dose level of GSK3352589 (either an increase or a decrease) will be made by the Medical Monitor and the investigator based on safety, tolerability and preliminary PK data obtained in at least 4 GSK3352589-treated subjects at the prior dose level. The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety or PK findings at a given dose level or to add cohorts to evaluate up to 2 additional dose levels or dosing regimens. The study procedures for these additional participants/cohorts will be the same as those described for other study participants/cohorts. If moderate or severe AEs are consistently observed across participants in a cohort or if unacceptable pharmacological effects, reasonably attributable to study treatment in the opinion of the investigator are observed in more than 1 participant in a cohort, than dose escalation will be temporarily halted and no further participants will be dosed until a full safety review of the study has taken place. Relevant reporting and discussion with Medical Monitor and the Ethics Committee will take place before resumption of dosing.

7.3. Method of Treatment Assignment

The randomization schedule will be computer generated by GSK. On Day 1, subjects will be assigned a unique number (randomization number) in ascending numerical order. The randomization number encodes the participant's assignment to a treatment arm or sequence, according to the randomization schedule generated prior to the study by the Statistics Department at GSK. Each participant will be dispensed blinded study treatment, labeled with his/her unique randomization number, throughout the study.

7.4. Blinding

This will be a double blind (sponsor unblind) study and the following will apply. GSK staff will be considered unblinded to treatments, though the number with access to treatment information will be minimized.

- The investigator or treating physician may unblind a subject's treatment assignment **only in the case of an emergency** OR in the event of a serious medical condition when knowledge of the study treatment is essential for the appropriate clinical management or welfare of the subject as judged by the investigator.
- Investigators have direct access to the subject's individual study treatment.
- It is preferred (but not required) that the investigator first contacts the Medical Monitor or appropriate GSK study personnel to discuss options **before** unblinding the subject's treatment assignment.
- If GSK personnel are not contacted before the unblinding, the investigator must notify GSK as soon as possible after unblinding, but without revealing the treatment assignment of the unblinded subject, unless that information is important for the safety of subjects currently in the study.
- The date and reason for the unblinding must be fully documented in the eCRF

A subject will be withdrawn if the subject's treatment code is unblinded by the investigator or treating physician. The primary reason for discontinuation (the event or condition which led to the unblinding) will be recorded in the eCRF.

- GSK's Global Clinical Safety and Pharmacovigilance (GCSP) staff may unblind the treatment assignment for any subject with an SAE. If the SAE requires that an expedited regulatory report be sent to one or more regulatory agencies, a copy of the report, identifying the subject's treatment assignment, may be sent to investigators in accordance with local regulations and/or GSK policy.

7.5. Preparation/Handling/Storage/Accountability

The investigator or designee must confirm appropriate temperature conditions have been maintained during transit for all study treatment received and any discrepancies are reported and resolved before use of the study treatment.

Only participants enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored

in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study treatment are provided in the Study Reference Manual.

- A Material Safety Data Sheet (MSDS)/equivalent document describing occupational hazards and recommended handling precautions either will be provided to the investigator, where this is required by local laws, or is available upon request from GSK.

Precaution will be taken to avoid direct contact with the study treatment. A Material Safety Data Sheet (MSDS) describing occupational hazards and recommended handling precautions will be provided to the investigator. In the case of unintentional occupational exposure notify the monitor, Medical Monitor and/or GSK study contact.

7.6. Treatment Compliance

Participants are dosed at the site and will receive study treatment directly from the investigator or designee, under medical supervision. The date and time of each dose administered in the clinic will be recorded in the source documents. The dose of study treatment and study participant identification will be confirmed at the time of dosing by a member of the study site staff other than the person administering the study treatment. Study site personnel will examine each participant's mouth to ensure that the study treatment was ingested.

7.7. Concomitant Therapy

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the participant is receiving at the time of enrolment or receives during the study must be recorded along with:

- reason for use
- dates of administration including start and end dates
- dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Participants must abstain from taking prescription or nonprescription drugs (including vitamins and dietary or herbal supplements) within 7 days (or 14 days if the drug is a potential enzyme inducer) or 5 half-lives (whichever is longer) before the start of study treatment until completion of the follow-up visit, unless, in the opinion of the investigator and GSK medical monitor, the medication will not interfere with the study.

Paracetamol, at doses of ≤ 2 grams/day, is permitted for use any time during the study. Other concomitant medication may be considered on a case-by-case basis by the investigator, in consultation with the Medical Monitor, if required.

7.8. Treatment after the End of the Study

Subjects will not receive any additional treatment from GSK after completion of the study because only healthy volunteers are eligible for study participation.

8. DISCONTINUATION CRITERIA

8.1. Discontinuation of Study Treatment

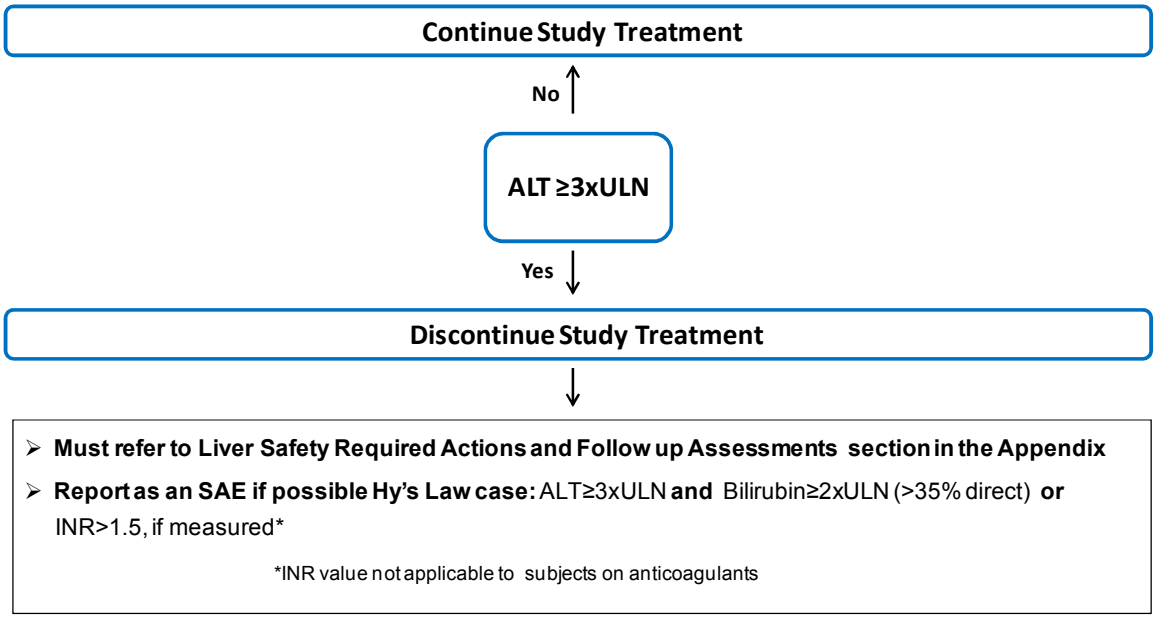
A subject may withdraw from study treatment at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural or administrative reasons. If a subject withdraws from the study, he/she may request destruction of any samples taken, and the investigator must document this in the site study records.

8.1.1. Liver Chemistry Stopping Criteria

Liver chemistry stopping and increased monitoring criteria have been designed to assure participant safety and evaluate liver event etiology.

Study treatment will be discontinued **for a participant** if liver chemistry stopping criteria are met:

Phase I Liver Chemistry Stopping Criteria – Liver Stopping Event Algorithm



Liver Safety Required Actions and Follow up Assessments Section can be found in Section 12.8.

8.1.2. QTc Stopping Criteria

- The QTcF correction formula *must* be used for *each individual subject* to determine eligibility for and discontinuation from the study. This formula may not be changed or substituted once the subject has been enrolled.
- The QTc should be based on averaged QTcF values of triplicate electrocardiograms obtained over a brief (e.g., 5-10 minute) recording period.

A subject that meets either bulleted criterion based on the average of triplicate ECG readings will be withdrawn from the study:

- QTcF > 500 msec,
- Change from baseline: QTcF >60 msec

See the SoA for data to be collected at the time of treatment discontinuation and follow-up and for any further evaluations that need to be completed.

8.2. Withdrawal from the Study

- A participant may withdraw from the study at any time at his/her own request, or may be withdrawn at any time at the discretion of the investigator for safety, behavioural, compliance or administrative reasons.

- If the participant withdraws consent for disclosure of future information, the sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a participant withdraws from the study, he/she may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.
- Refer to the SoA for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed.

8.3. Lost to Follow Up

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

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

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow up, the investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record.
- Should the participant continue to be unreachable, he/she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

9. STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA.
- Protocol waivers or exemptions are not allowed
- Immediate safety concerns should be discussed with the sponsor immediately upon occurrence or awareness to determine if the participant should continue or discontinue study treatment.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential participants meet all eligibility criteria. The investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

- Procedures conducted as part of the participant's routine clinical management (eg, blood count) and obtained before signing of ICF may be utilized for screening or baseline purposes provided the procedure met the protocol-specified criteria and was performed within the time frame defined in the SoA.
    - The maximum amount of blood collected from each participant over the duration of the study, including any extra assessments that may be required, will not exceed 470 mL.
    - Repeat or unscheduled samples may be taken for safety reasons or for technical issues with the samples.

9.1. Adverse Events

The definitions of an AE or SAE can be found in Section 12.6.

The investigator and any designees are responsible for detecting, documenting, and reporting events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study treatment or the study, or that caused the participant to discontinue the GSK3352589 (see Section 12.6).

9.1.1. Time Period and Frequency for Collecting AE and SAE Information

- All SAEs will be collected from the signing of the ICF until the follow-up visit at the time points specified in the SoA (Section 2).
- All AEs will be collected from the start of treatment until the follow-up visit at the time points specified in the SoA (Section 2).
- Medical occurrences that begin before the start of study treatment but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the case report form (CRF) not the AE section.
- All SAEs will be recorded and reported to the sponsor or designee within 24 hours, as indicated in Section 12.6. The investigator will submit any updated SAE data to the sponsor within 24 hours of it being available.
- Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE, including a death, at any time after a participant has been discharged from the study, and he/she considers the event to be reasonably related to the study treatment or study participation, the investigator must promptly notify the sponsor.
- The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 12.6.

9.1.2. Method of Detecting AEs and SAEs

Care will be taken not to introduce bias when detecting AE and/or SAE. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrence.

9.1.3. Follow-up of AEs and SAEs

After the initial AE/SAE report, the investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until the event is resolved, stabilized, otherwise explained, or the participant is lost to follow-up (as defined in Section 8.3). Further information on follow-up procedures is given in Section 12.6.

9.1.4. Regulatory Reporting Requirements for SAEs

- Prompt notification by the investigator to the sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.
- The sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation. The sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and sponsor policy and forwarded to investigators as necessary.
- An investigator who receives an investigator safety report describing a SAE or other specific safety information eg, summary or listing of SAE) from the sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

9.1.5. Pregnancy

- Female subjects of child bearing potential are excluded from this study.
- Details of all pregnancies in female participants and female partners of male participants will be collected for pregnancies occurring after the start of study treatment and until 7 days after the last dose.
- If a pregnancy is reported, the investigator should inform GSK within 24 hours of learning of the pregnancy and should follow the procedures outlined in Section 12.7.
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

9.2. Treatment of Overdose

For this study, any dose of GSK3352589 greater than 400 mg within a 24-hour time period ± 1 hour will be considered an overdose.

GSK does not recommend specific treatment for an overdose

In the event of an overdose the investigator should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the participant for AE/SAE and laboratory abnormalities until GSK3352589 can no longer be detected systemically (at least 7 days for GSK3352589).
3. Obtain a plasma sample for PK analysis within 24 hours from the date of the last dose of study treatment if requested by the Medical Monitor (determined on a case-by-case basis).
4. Document the quantity of the excess dose as well as the duration of the overdosing in the CRF.

Decisions regarding dose interruptions or modifications will be made by the investigator in consultation with the Medical Monitor based on the clinical evaluation of the participant.

9.3. Safety Assessments

Planned time points for all safety assessments are provided in the SoA. Additional time points for safety tests such as vital signs, physical exams and laboratory safety tests may be added during the course of the study based on newly available data to ensure appropriate safety monitoring.

9.3.1. Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the Skin, Cardiovascular, Respiratory, Gastrointestinal and Neurological systems. Height and weight will also be measured and recorded. Please refer to SoA as to when assessments are required.
- A brief symptom directed physical examination will include, at a minimum, assessments of the skin, lungs, cardiovascular system, and abdomen (liver and spleen).
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

9.3.2. Vital Signs

- Vital signs will be measured in a semi-supine position after 5 minutes rest and will include temperature, systolic and diastolic blood pressure, and pulse rate.
- Three readings of blood pressure and pulse will be taken. The first reading should be rejected. The second and third readings should be averaged to give the measurement to be recorded in the CRF

9.3.3. Electrocardiograms

- Triplicate 12-lead ECGs will be obtained at each timepoint during the study using an ECG machine that automatically calculates the heart rate and measures

PR, QRS, QT, and QTc intervals. Refer to Section 8.1.2 for QTc withdrawal criteria and additional QTc readings that may be necessary

- At each time point at which triplicate ECG are required, 3 individual ECG tracings should be obtained as closely as possible in succession, but no more than 2 minutes apart. The full set of triplicates should be completed in less than 4 minutes. The postdose timepoints for ECGs may be changed if PK data indicate an earlier or later tmax.

9.3.4. Clinical Safety Laboratory Assessments

- Refer to Section 12.3 for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 7 days after the last dose of study treatment should be repeated until the values return to normal or baseline or are no longer considered significantly abnormal by the investigator or medical monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the investigator, the etiology should be identified and the sponsor notified.
- All protocol-required laboratory assessments, as defined in Section 12.3, must be conducted in accordance with the laboratory manual and the SoA.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution’s local laboratory require a change in participant management or are considered clinically significant by the investigator (eg, SAE or AE or dose modification), then the results must be recorded in the CRF.

9.3.5. Bristol Stool Form Scale

The BSFS [Lewis, 1997] which describes 7 types of stool, will be used by the subject during the study. Please refer to Section 12.4.

9.4. Pharmacokinetics

9.4.1. Sample Collection

9.4.1.1. Blood sample collection

Blood samples for PK analysis of GSK3352589 will be collected at the time points indicated in Section 2, Schedule of Activities. The actual date and time of each blood

sample collection will be recorded. The timing of PK samples may be altered and/or PK samples may be obtained at additional time points to ensure thorough PK monitoring.

Collection details, Processing, storage and shipping procedures are provided in the SRM.

9.4.1.2. Urine Sample Collection

Urine samples to aid in the structural identification of metabolites of GSK3352589 will be collected at the timepoints listed in Schedule of Activities, Section 2. Details of urine sample processing, storage and shipping procedures are provided in the SRM.

9.4.2. Sample Analysis

Plasma (PK) analysis will be performed under the control of PTS-IVIVT-BIB, GlaxoSmithKline, the details of which will be included in the SRM. Concentrations of GSK3352589 will be determined in plasma samples using the currently approved bioanalytical methodology. Raw data will be archived at the bioanalytical site (detailed in the SRM).

Plasma samples provided for metabolite analysis and any residual PK samples from the highest dose cohort in Part B (Days 1 and 14) will be analyzed for compound-related metabolites under a separate PTS-PD-Global Spectroscopy, GlaxoSmithKline protocol. In addition, urine samples will be collected pre-dose and over the post-dose time intervals 0-24 hours (Days 1 and 14) and qualitatively analyzed for metabolites under a separate PTS-PD-Global Spectroscopy, GlaxoSmithKline protocol from the highest dose cohort only.

Genetic analyses will not be performed on any samples.

9.4.3. Pharmacokinetic Analyses

Pharmacokinetic analysis will be the responsibility of the Clinical Pharmacology Sciences department within GlaxoSmithKline or designee. Plasma GSK3352589 concentration-time data will be analyzed by non-compartmental methods with the most current version of Phoenix WinNonlin. Final calculations will be based on the actual sampling times recorded during the study.

From the plasma concentration time data following single doses in Part A, the following pharmacokinetics parameters will be determined, as data permit, Cmax, Tmax, AUC(0-last), AUC(0-24), AUC(0-infinity), t1/2 or MRT, Tlast, AUC extrapolated to infinity (AUCPEO), and Tlag for the food effect comparisons only.

From the plasma concentration time data following repeat doses in Part B, the following PK parameters will be determined, as data permit for both Days 1 and 14: maximum observed plasma concentration (Cmax) for both the dosing intervals and the 24 hour daily interval, Tmax for both dosing intervals and 24 hour daily interval, AUCs for both the dosing intervals (0-10h) or (0-24h) for both QD and BID repeat dose regimens. Tlast parameter will be included in the reporting. The Cmax and AUC parameters will be used

to assess dose proportionality in both Part A and B and accumulation in Part B as data permits.

9.5. Pharmacodynamic Biomarkers

Subjects in Part B will undergo PD testing of total GLP-1 and total PYY levels after administration of a breakfast mixed-meal (PD meal) challenge on Day -1 (baseline), Day 1 and Day 14 at the end of the treatment period according to Section 2. The timing of PD samples may be altered and/or PD samples may be obtained on additional days or at additional time points. The samples for peptide analyses must be handled with extra care due to ex vivo degradation. Details on collection, processing, storage and shipping of blood samples are provided in the SRM.

9.6. Genetics

Genetics are not evaluated in this study.

10. STATISTICAL CONSIDERATIONS

10.1. Sample Size Determination

A sufficient number of subjects will be screened to enroll 16 subjects for Part A and 48 subjects (8 subjects/cohort) for Part B for a total of 64 randomized subjects. Sample sizes are based on feasibility. No formal power calculations were performed, and no statistical sample size re-estimation or adjustment will be used.

10.2. Populations for Analyses

For purposes of analysis, the following populations are defined:

Population	Description
Safety	All randomized participants who receive at least one dose of study medication. Participants will be analyzed according to the treatment they actually received.
PK Concentration	All subjects for whom a pharmacokinetic sample was obtained.
PK Parameter	All subjects in the PK Concentration Population who receive at least one active dose of GSK3352589 and provide pharmacokinetic parameters.
PD	All subjects who receive at least one dose of study drug with a baseline value and at least one postprandial PD biomarker value.

10.3. Statistical Analyses

Safety and tolerability will be presented in tabular and/or graphical format and summarized descriptively. No hypotheses will be tested. Listings of adverse events and point estimates and 90% confidence intervals for numerical endpoints will be produced. BSFS will be summarized by frequency and proportion of stool types by subjects and by treatment arm.

Pharmacokinetic data will be presented in graphical and/or tabular form and will be summarized descriptively. Descriptive statistics (n, arithmetic mean, standard deviation, %CV, minimum, median, and maximum) will be calculated for all pharmacokinetic parameters by treatment. For log-transformed variables, geometric mean, 95% confidence interval, and %CVb (100 * √ (exp(SD2) -1)) will be provided, where the SD is the standard deviation of log-transformed data. All pharmacokinetic data will be stored in the Archives, GlaxoSmithKline Pharmaceuticals, R&D. Data permitting, the PK parameter data will be presented descriptively (e.g. n, arithmetic mean, median, minimum, maximum) by treatment and part. Further summaries, analyses, and/or graphs may be produced as appropriate to the data.

Statistical analyses of the pharmacokinetic data will be the responsibility of Clinical Statistics, GlaxoSmithKline or designee. In Part A, dose proportionality of GSK3352589 will be analyzed by using appropriate power and ANOVA models. Food effect analyses will be done according to the FDA guidance standards. In Part B, the Cmax and AUC parameters will be used to assess accumulation and dose proportionality.

Analyses of PD data will be the responsibility of Clinical Statistics, GlaxoSmithKline or designee. PD data for total GLP-1 and total PYY will be presented in graphical and/or tabular form and will be summarized descriptively.

If data permit, the relationship between GSK3352589 and postprandial levels will be explored. PK/PD analyses will be performed to determine if relationship(s) between dose and/or GSK3352589 concentrations and postprandial concentrations of GLP-1 and PYY levels exist. Graphical investigations will be initially explored and appropriate linear and non-linear models may be evaluated.

Biotransformation of GSK3352589 in plasma and urine will also be evaluated for the identification of any compound-derived metabolite(s).

10.3.1. Other Analyses

Pharmacokinetic, pharmacodynamic, and biomarker exploratory analyses will be described in the reporting and analysis plan.

10.3.2. Interim Analyses

No formal statistical interim analysis is planned for this study. Safety, tolerability, and pharmacokinetic data will be reviewed prior to each dose-escalation and prior to all within subject escalations.

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12. APPENDICES

12.1. Appendix 1: Abbreviations and Trademarks

AE	Adverse Event
ALT	Alanine Transferase
ANOVA	Analysis of variance
AST	Aspartate Transferase
AUC	Area Under the Curve
AUCtau	Area under the plasma concentration-time curve over the dosing interval (0-4), (0-10), and (0-24) hours
AUClastor AUC (0-t)	Area under the plasma concentration-time curve from time zero to last measurable time point
BCS	Biopharmaceutical classification system
BID	Twice a Day or twice daily
BSA	Body Surface Area
BSFS	Bristol Stool Form Scale
BUN	Blood Urea Nitrogen
CDER	Center for Drug Evaluation and Research
CL/F	Clearance
CLs	Systemic clearance
Cmax	Maximum Observed Plasma Concentration
CONSORT	Consolidated Standards of Reporting Trials
CNS	Central Nervous System
CTT	Colonic Transit Time
CRF	Case Report Form
CV	Cardiovascular
CYP	Cytochrome P450
DDI	Drug-Drug Interaction
e.g.	exempli gratia (for example)
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ENS	Enteric Nervous System
F	Bioavailability
FDA	(US) Food and Drug Administration
FSH	Follicle Stimulating Hormone
FTiH	First Time in Human
g	gram
GCP	Good Clinical Practice
GCSP	Global Clinical Safety and Pharmacovigilance
GET	Gastric Emptying Time
GI	Gastrointestinal
GSK	GlaxoSmithKline
h	hour
HbsAg	Hepatitis B Surface Antigen
hCG	Human Chorionic Gonadotropin

HED	Human Equivalent Dose
Hep B	Hepatitis B
Hep C	Hepatitis C
hERG	Human ether-a-go-go-related gene
HIV	Human Immunodeficiency Virus
HRT	Hormone Replacement Therapy
IB	Investigator's Brochure
IBS	Irritable Bowel Syndrome
IBS-C	Irritable Bowel Syndrome Constipation
IBS-D	Irritable Bowel Syndrome Diarrhea
IBS-M	Irritable Bowel Syndrome Mixed
IC50	Half Maximal Inhibitory Concentration
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
INR	International Normalized Ratio
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
Ka	Absorption rate constant
kg	Kilogram
Ki	Inhibitory Constant
MABEL	Minimum anticipated biological effect level
MCH	Mean Corpuscular Hemoglobin
MCV	Mean Corpuscular Volume
MedDRA	Medical Dictionary for Regulatory Activities
mg	milligram
mL	milliliter
MLA	Mouse Lymphoma Assay
MRTlast	Mean residence time up to last measurable concentration
MSDS	Material Safety Data Sheet
msec	Millisecond
ng/g	Nanogram per gram
ng/mg	Nanogram per milligram
nM	Nanometer
NOAEL	No Observed Adverse Effect Level
PAD	Pharmacological active dose
PD	Pharmacodynamic
PK	Pharmacokinetic
PI	Percentage Interval
PV	Pharmacovigiliance
QTc	Corrected QT Interval
QTcB	Corrected QT Interval to Bazett's formula
QTcF	Corrected QT Interval to Fridericia's formula
RAP	Report and Analysis Plan
RBC	Red Blood Cell
RET	REarranged during Transfection

RSA	Relative surface area
SAE	Serious Adverse Event
SBTT	Small Bowel Transit Time
SD	Standard Deviation
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOA	Schedule of Activities
SOP	Standard Operating Procedure
SRM	Study Reference Manual
T1/2	Apparent Terminal Phase Half-Life
Tmax	Time to Maximum Observed Plasma Concentration
µg	Microgram
ULN	Upper Limit of Normal
V/F	Volume of distribution
WBC	White Blood Cell
WGTT	Whole Gut Transit Time
WMC	Wireless Motility Capsule

Trademark Information

Trademarks of the GlaxoSmithKline group of companies
ADVAIR

Trademarks not owned by the GlaxoSmithKline group of companies
Chiron RIBA
Phoenix WinNonlin
SAS

12.2. Appendix 2: Human Pharmacodynamic Prediction Methods

A systems pharmacology model was developed for rats and then scaled to humans for GSK3352589 in GI tract with a focus on colon lumen and colon tissue concentrations. Additional methods based on allometry concepts (body weight, BSA, and intestine surface areas) were applied for additional support and to facilitate expansion and account for error in the predicted dose range for this study. The dose range proposed for minimal pharmacological effect may be 5 mg once daily (2 mg twice daily) to 130 mg once or twice daily. The dose range for maximum pharmacological effect is predicted to be 5 mg to 440 mg. It is unknown if these doses are best given once or twice daily, but twice daily dosing should provide more sustained tissue concentrations per day. The rat IBS visceral hypersensitivity model indicates dosing to steady state tissue concentrations is required and that twice daily dosing is best in rats. Efficacy with the rat PD colon hypersensitivity model correlates with a steady state colon tissue concentration of approximately 1218 ng/g (10 mg/kg for 3.5 days BID) and this target tissue concentration was assumed to be the same for human colon tissue.

12.2.1. Systems pharmacology model of human colon tissue concentrations

Rat plasma profiles show little or no correlation with the rat model PD response. This presented a unique challenge as many established methods for determining dose/exposure response for a predetermined target concentration have been based on plasma drug concentrations. As colon tissue concentrations were determined to be the best predictor of patient response, it was determined a custom systems pharmacology modeling effort would be appropriate to address this challenge. A mechanistic model was developed to predict colon tissue concentrations as a function of colon content drug concentration. Development began with the determination of gut transit times, gut thickness, volumes and surfaces areas for rats and humans from the literature. Time course data from rat gut content and tissue concentrations were first used to estimate drug specific parameters, than once identified were fixed for subsequent human predictions. The model was used to simulate colon tissue concentrations for doses between 2 mg and 400 mg. Colon tissue time profile predictions were simulated to determine the time and duration above the target tissue concentration of 1218 ng/g following once daily (Figure 2) and twice daily (Figure 3). Following once daily oral doses of 7.5 mg and 10 mg and twice daily repeat dosing with 2 mg, 3 mg, and 5 mg, the colon tissue concentration remains above or near the target concentration of 1218 ng/g for the entire dosing interval. The duration of exposure needed or the time above 1218 ng/g is unknown.

Figure 2 Predicted human colon tissue concentration-time profiles following once daily dosing for four days ranging from 1 mg to 10 mg

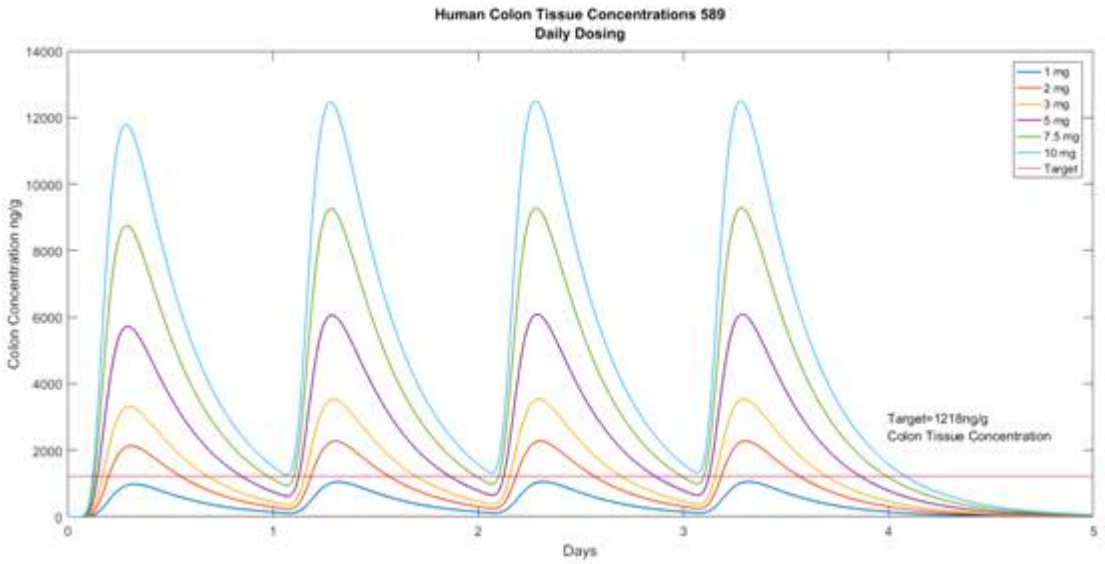
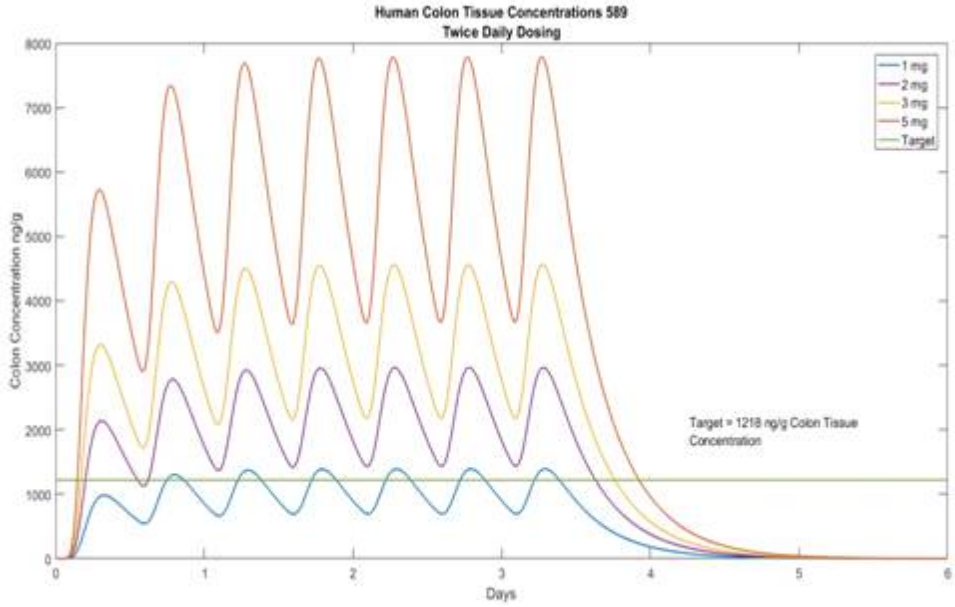


Figure 3 Predicted human colon tissue concentration-time profiles following twice daily dosing for four days ranging from 1 mg to 5 mg



12.2.2. Human Equivalent Dose (HED) Prediction Method using FDA guidance with and without small and large intestine surface area correction

This HED method is generally recommended for estimating safe starting doses in FTiH studies (FDA - CDER 2005 guidance), herein the conversion factors have been applied to the pharmacologically active doses (PAD). The HEDs are shown in Table 9. HED is

essentially a body surface area (BSA) correction. Because the rat and human have similar in vitro enzyme inhibition potency estimates, a potency adjustment was not needed. Further correction was applied to the HED for small intestine surface area based on the principle that more small intestine surface area will result in more absorption of drug in humans. It has been reported that the ratio of the human small intestine surface area normalized for BSA is 111. The same ratio for rat is 25. This comparison reveals that relative surface area of the human small intestine (RSA) is 4.4 times that of the rat [DeSesso, 2001]. The HED was divided by 4.4 and referred to as HED corrected for small intestine RSA and is considered a very conservative estimate.

In addition, correction was applied to the HED for colon surface area based on the principle there is less large intestine surface area in human compared to rat. It has been reported that the ratio of the human large intestine surface area normalized for BSA is 0.19. The same ratio for rat is 0.85. This comparison reveals that the large intestine relative surface area of the human is 0.22 times that of the rat [DeSesso, 2001]. The HED was divided by 0.22 and referred to as HED corrected for large intestine RSA and is considered the more aggressive estimate.

Using these prediction methods, human doses anticipated to have minimum pharmacological effect are predicted to be between 7 mg and 136 mg for a 60 kg human following repeat dosing to steady state. The doses anticipated to achieve maximum pharmacological effect are predicted to be between 23 mg and 454 mg for a 60 kg human.

Table 8 Human Dose Predictions

Rat (0.25kg)	**HED (60 kg)**		**HED corrected for small intestine RSA (rat to human)**	**HED corrected for large intestine RSA (rat to human)**
3 mg/kg* Minimal effect; Rat visceral hypersensitivity model	0.48 mg/kg	~30mg	~7 mg	~136 mg
10mg/kg* Maximal effect; Rat visceral hypersensitivity model	1.61 mg/kg	~100 mg	~23 mg	~454 mg

*dosed twice daily for 3.5 days (7 doses) to steady state

12.2.3. Human Dose Prediction for of GSK3352589 Adjusted for Colon Content and Gut Weight

The minimally effective dose in the IBS-hypersensitivity rat model was 3 mg/kg and the maximally effective dose was 10 mg/kg. The estimated bioavailability is 0.18% for rat and approximately 0.16% for human; therefore 99.82% and 99.84% of the dose remains in the GI tract from oral to elimination.

The calculations below assume that the amount of GSK3353589 per gram of whole GI tract tissue at doses linked to efficacy rat model will be the same amounts and doses needed for humans. Assuming the target colon content for human is between 115,185 ng/g and 384,000 ng/g, the corresponding human doses are 122 mg and 406 mg (Table 9).

Table 9 Method for human colon content and gut weight estimation

Parameter	Rat (0.25kg)	Rat (0.25kg)	Human (60 kg)	Human (60 kg)
Dosed to steady state	3mg/kg	10mg/kg	2.03mg/kg	6.77mg/kg
Dose	0.75 mg or 750 µg	2.5mg or 2500 µg	122 mg	406 mg
≈ 99.82% of dose remaining in gut contents	748.7 µg	2496 µg	-	-
≈ 99.84% of dose remaining in gut contents	-	-	121,635 µg	405,504 µg
Whole GI tract (grams or mL) (stomach, small and large intestine)[1]	6.5 g	6.5 g	1056 g	1056 g
Calculated amount of drug in rat colon contents	115,185 ng/g	384,000 ng/g	-	-
Targeted amount of drug in human colon contents	-	-	115,185 ng/g	384,000 ng/g

NOTES :

1. Physiological parameter values for PBPK models: a report by the international life sciences institute risk science institute. U.S. Environmental Protection Agency, Office of Health and Environmental Assessment, 1994.

12.3. Appendix 3: Clinical Laboratory Tests

- The tests detailed in Table 10 will be performed by the local laboratory.
- Protocol-specific requirements for inclusion or exclusion of participants are detailed in Section 6 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the investigator or required by local regulations.

Table 10 Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters			
Hematology	Platelet Count RBC Count Hemoglobin Hematocrit	RBC Indices: MCV MCH	WBC count with Differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils	
Clinical Chemistry[1]	BUN	Potassium	Aspartate Aminotransferase (AST)/ Serum Glutamic-Oxaloacetic Transaminase (SGOT)	Total and direct bilirubin
	Creatinine	Sodium	Alanine Aminotransferase (ALT)/ Serum Glutamic-Pyruvic Transaminase (SGPT)	Total Protein
	Glucose - nonfasting]	Calcium	Alkaline phosphatase	Albumin
Routine Urinalysis	• Specific gravity • pH, glucose, protein, blood, ketones, [bilirubin, urobilinogen, nitrite, leukocyte esterase] by dipstick • Microscopic examination (if blood or protein is abnormal)			
Other Screening Tests	• HIV • Hepatitis B (HBsAg) • Hepatitis C (Hep C antibody) • FSH and estradiol (as needed in women of non-child bearing potential only) • Cotinine test • Alcohol and drug screen (to include at minimum: amphetamines, barbiturates, cocaine, opiates, cannabinoids and benzodiazepines)			

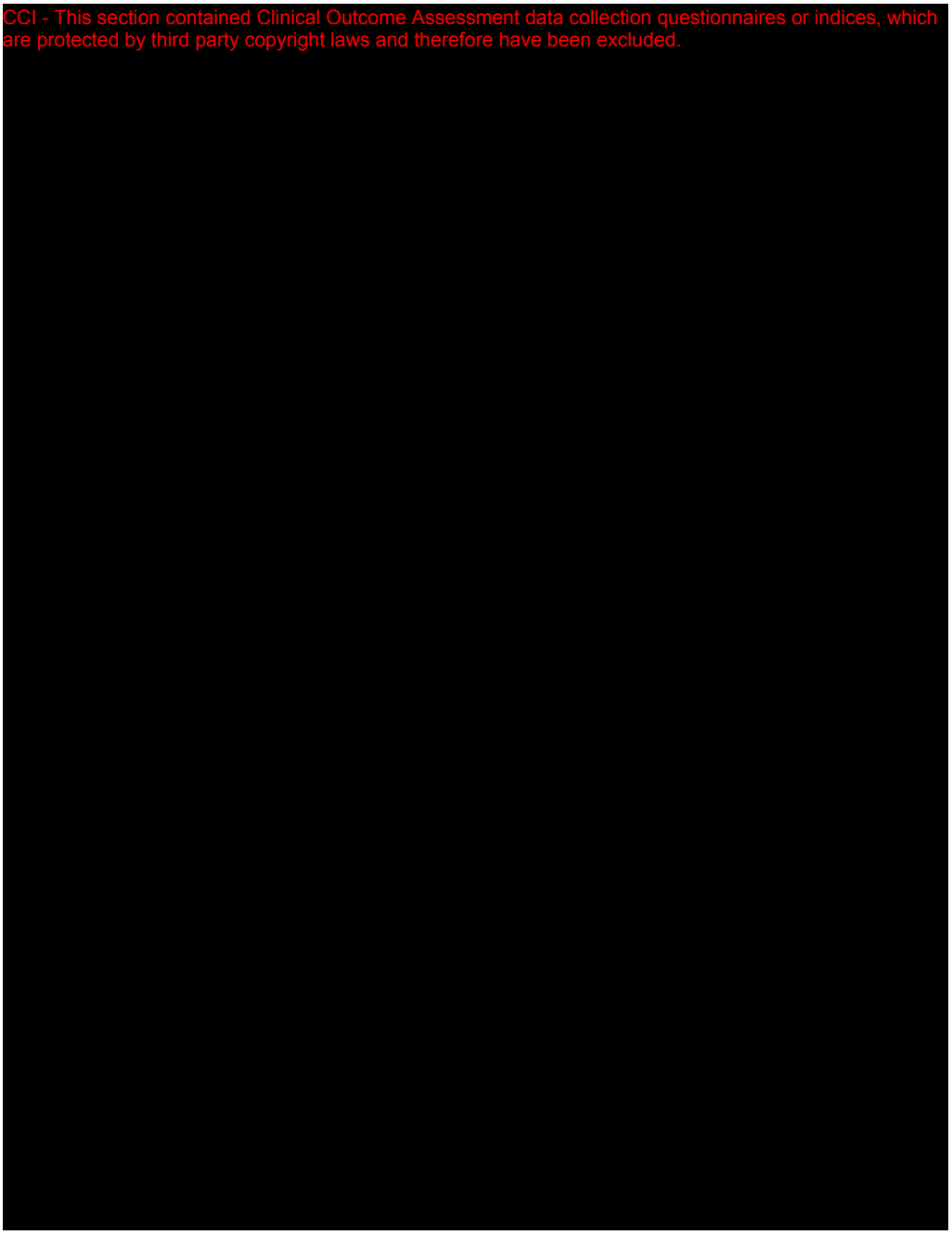
Laboratory Assessments	Parameters
	• Serum hCG Pregnancy test (Screening and follow up) and urine pregnancy test on Day -1.

NOTES :

1. Details of liver chemistry stopping criteria and required actions and follow-up assessments after liver stopping or monitoring event are given in Section 8.1 and Section 12.8. All events of ALT ≥3 × upper limit of normal (ULN) and bilirubin ≥2 × ULN (>35% direct bilirubin) or ALT ≥3 × ULN and international normalized ratio (INR) >1.5, if INR measured, which may indicate severe liver injury (possible Hy's Law), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis).
2. Local urine testing will be standard for the protocol unless serum testing is required by local regulation or IRB/IEC.

12.4. Appendix 4: Bristol Stool Form Scale

CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.



12.5. Appendix 5: Study Governance Considerations

Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable ICH Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IEC/IRB approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.
- The investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC
    - Notifying the IRB/IEC of SAE or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

Informed Consent Process

- The investigator or his/her representative will explain the nature of the study to the participant or his/her legally authorized representative and answer all questions regarding the study.
- Participants must be informed that their participation is voluntary. Participants or their legally authorized representative will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the participant was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.

- Participants must be re-consented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the participant or the participant's legally authorized representative.
- Participants who are rescreened are required to sign a new ICF.

Data Protection

- Participants will be assigned a unique identifier by the sponsor. Any participant records or datasets that are transferred to the sponsor will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.
- The participant must be informed that his/her personal study-related data will be used by the sponsor in accordance with local data protection law. The level of disclosure must also be explained to the participant.
- The participant must be informed that his/her medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the investigator agrees to submit all manuscripts or abstracts to the sponsor before submission. This allows the sponsor to protect proprietary information and to provide comments.
- The sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

Dissemination of Clinical Study Data

- Tabular study results will be posted on the US National Institutes of Health's website ww.clintrials.gov and other publically accessible sites.

Data Quality Assurance

- All participant data relating to the study will be recorded on printed or electronic CRF unless transmitted to the sponsor or designee electronically (eg, laboratory data). The investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.

- The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- The sponsor or designee is responsible for the data management of this study including quality checking of the data.
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of participants are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.
- Records and documents, including signed ICF, pertaining to the conduct of this study must be retained by the investigator for 25 years from the issue of the final Clinical Study Report (CSR)/ equivalent summary unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the sponsor. No records may be transferred to another location or party without written notification to the sponsor.

Source Documents

- Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected. Source documents are filed at the investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The investigator may need to request previous medical records or transfer records, depending on the study. Also, current medical records must be available.
- Definition of what constitutes source data can be found in the study reference manual (SRM).

Study and Site Closure

GSK or its designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of GSK. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the sponsor or investigator may include but are not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures, or GCP guidelines

- Inadequate recruitment of participants by the investigator
- Discontinuation of further study treatment development

12.6. Appendix 6: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

Definition of AE

AE Definition
• An AE is any untoward medical occurrence in a clinical study participant, temporally associated with the use of a study treatment, whether or not considered related to the study treatment. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a study treatment.

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study treatment administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study treatment or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae.

Events NOT Meeting the AE Definition
• Any clinically significant abnormal laboratory findings or other abnormal safety assessments which are associated with the underlying disease, unless judged by the investigator to be more severe than expected for the participant's condition. • The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the participant's condition. • Medical or surgical procedure (eg, endoscopy, appendectomy): the condition that leads to the procedure is the AE. • Situations in which an untoward medical occurrence did not occur (social and/or

convenience admission to a hospital).

- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.

Definition of SAE

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

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

a. Results in death

b. Is life-threatening

The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant 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 were more severe.

c. Requires inpatient hospitalization or prolongation of existing hospitalization

In general, hospitalization signifies that the participant has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AE. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.

d. Results in persistent disability/incapacity

- The term disability means a substantial disruption of a person's ability to conduct normal life functions.
- This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect

f. Other situations:

- Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the participant or may require medical or surgical intervention to prevent

one of the other outcomes listed in the above definition. These events should usually be considered serious.

Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

Recording AE and SAE

AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (eg, hospital progress notes, laboratory, and diagnostics reports) related to the event.
- The investigator will then record all relevant AE/SAE information in the CRF.
- It is **not** acceptable for the investigator to send photocopies of the participant's medical records to GSK in lieu of completion of the GSK /AE/SAE CRF page.
- There may be instances when copies of medical records for certain cases are requested by GSK. In this case, all participant identifiers, with the exception of the participant number, will be redacted on the copies of the medical records before submission to GSK.
- The investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

Assessment of Intensity

The investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories:

- Mild: An event that is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
- Moderate: An event that causes sufficiently discomfort and interferes with normal everyday activities.
- Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with an SAE. Severe is a category utilized for rating the intensity of an event; and both AE and SAE can be assessed as severe.

  An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe.

Assessment of Causality

- The investigator is obligated to assess the relationship between study treatment and each occurrence of each AE/SAE as follows: unrelated, likely unrelated, possibly

related, probably related and related.

- A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out.
- The investigator will use clinical judgment to determine the relationship.
- Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study treatment administration will be considered and investigated.
- The investigator will also consult the Investigator's Brochure (IB) and/or Product Information, for marketed products, in his/her assessment.
- For each AE/SAE, the investigator **must** document in the medical notes that he/she has reviewed the AE/SAE and has provided an assessment of causality.
- There may be situations in which an SAE has occurred and the investigator has minimal information to include in the initial report to GSK. However, **it is very important that the investigator always make an assessment of causality for every event before the initial transmission of the SAE data to** GSK**.**
- The investigator may change his/her opinion of causality in light of follow-up information and send an SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Follow-up of AE and SAE

- The investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by GSK to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the investigator will provide GSK with a copy of any post-mortem findings including histopathology.
- New or updated information will be recorded in the originally completed CRF.
- The investigator will submit any updated SAE data to GSK within 24 hours of receipt of the information.

Reporting of SAE to GSK

SAE Reporting to GSK via Electronic Data Collection Tool

- The primary mechanism for reporting SAE to GSK will be the electronic data

collection tool.

- If the electronic system is unavailable for more than 24 hours, then the site will use the paper SAE data collection tool (see next section).
- The site will enter the SAE data into the electronic system as soon as it becomes available.
- The investigator or medically-qualified sub-investigator must show evidence within the eCRF (e.g., check review box, signature, etc.) of review and verification of the relationship of each SAE to IP/study participation (causality) within 72 hours of SAE entry into the eCRF.
- After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data.
- If a site receives a report of a new SAE from a study participant or receives updated data on a previously reported SAE after the electronic data collection tool has been taken off-line, then the site can report this information on a paper SAE form (see next section) or to the medical monitor by telephone.
- Contacts for SAE reporting can be found in the SRM and at the beginning of this protocol on the Sponsor/Medical Monitor Contact Information page.

12.7. Appendix 7: Contraceptive Guidance and Collection of Pregnancy Information

Definitions

Woman of Childbearing Potential (WOCBP)

Female participants

Female participants of childbearing potential are NOT eligible to participate.

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below)

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with ONE of the following:

- Documented hysterectomy
- Documented bilateral salpingectomy
- Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's: review of participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Females on HRT and whose menopausal status is in doubt will be required to use one of the non-hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance

Male participants

- Male participants with female partners of child-bearing potential are eligible to participate if they agree to ONE of the following during the protocol-defined time frame in Section 6.1 (up to 7 days after administration of the last dose of study drug):

- Are abstinent from penile-vaginal intercourse as their usual and preferred lifestyle (abstinent on a long term and persistent basis) and agree to remain abstinent
- Agree to use a male condom plus an additional method of contraception with a failure rate of <1% per year as described in Table 11 when having penile-vaginal intercourse with a woman of childbearing potential

- Men with a pregnant or breastfeeding partner must agree to remain abstinent from penile-vaginal intercourse or use a male condom during each episode of penile penetration for 7 days after study completion or from last dose
- Refrain from donating sperm for duration of study and for 7 days after study completion or from last dose

For female partners of males participating in the study, highly effective contraceptive methods are described in the table below.

Table 11 Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent [a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen- and progestogen-containing) hormonal contraception associated with inhibition of ovulation[b] • oral • intravaginal • transdermal
Progestogen-only hormonal contraception associated with inhibition of ovulation[b] • injectable
Highly Effective Methods That Are User Independent
• Implantable progestogen-only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • bilateral tubal occlusion
Vasectomized partner *(A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.)*
Sexual abstinence (*Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study drug. The*

reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.)

NOTES:

a. Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants in clinical studies.

b. Hormonal contraception may be susceptible to interaction with the study drug, which may reduce the efficacy of the contraceptive method. In this case two highly effective methods of contraception should be utilized during the treatment period and for at least 7 days after the last dose of study treatment

Collection of Pregnancy Information

Male participants with partners who become pregnant

- Investigator will attempt to collect pregnancy information on any male participant's female partner of a male study participant who becomes pregnant while participating in this study. This applies only to participants who receive study treatment.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the investigator will record pregnancy information on the appropriate form and submit it to GSK within 24 hours of learning of the partner's pregnancy.
- Partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to GSK
- Generally, follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for procedure.

Female Participants who become pregnant

Female participants who can become pregnant are not eligible to participate, however any female subject who becomes pregnant while participating will be withdrawn from the study.

- Investigator will collect pregnancy information on any female participant, who becomes pregnant while participating in this study.
- Information will be recorded on the appropriate form and submitted to GSK within 24 hours of learning of a participant's pregnancy.
- Participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow up information on participant and neonate, which will be forwarded to GSK Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date.
- Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE.

- A spontaneous abortion is always considered to be an SAE and will be reported as such.
- Any SAE occurring as a result of a post-study pregnancy which is considered reasonably related to the study treatment by the investigator, will be reported to GSK as described in above. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

12.8. Appendix 8: Liver Safety: Required Actions and Follow-up Assessments

Phase I Liver chemistry stopping criteria have been designed to assure subject safety and to evaluate liver event etiology.

Phase I liver chemistry stopping criteria and required follow up assessments

<table>
<tr><th colspan="2">Liver Chemistry Stopping Criteria</th></tr>
<tr><td>ALT-absolute</td><td>ALT≥3xULN
If ALT≥3xULN AND bilirubin[1,2] ≥ 2xULN (>35% direct bilirubin) or INR >1.5, Report as an SAE.
See additional Actions and Follow Up Assessments listed below</td></tr>
<tr><th colspan="2">Required Actions and Follow up Assessments</th></tr>
<tr><th>Actions</th><th>Follow Up Assessments</th></tr>
<tr><td>• Immediately discontinue study treatment
• Report the event to GSK within 24 hours
• Complete the liver event CRF, and complete an SAE data collection tool if the event also meets the criteria for an SAE[2]
• Perform liver event follow up assessments
• Monitor the subject until liver chemistries resolve, stabilise, or return to within baseline (see MONITORING below)
MONITORING:
If ALT≥3xULN AND bilirubin ≥ 2xULN or INR >1.5
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within 24 hrs
• Monitor subjects twice weekly until liver chemistries resolve, stabilise or return to within baseline
• A specialist or hepatology consultation is recommended
If ALT≥3xULN AND bilirubin < 2xULN and INR ≤1.5:</td><td>• Viral hepatitis serology[3]
• Obtain INR and recheck with each liver chemistry assessment until the transaminases values show downward trend
• Obtain blood sample for pharmacokinetic (PK) analysis, obtained up to 5 days of last dose[4]
• Serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH).
• Fractionate bilirubin, if total bilirubin ≥ 2xULN
• Obtain complete blood count with differential to assess eosinophilia
• Record the appearance or worsening of clinical symptoms of liver injury, or hypersensitivity, on the AE report form
• Record use of concomitant medications on the concomitant medications report form including acetaminophen, herbal remedies, other over the counter medications.
• Record alcohol use on the liver event alcohol intake case report form
If ALT≥3xULN AND bilirubin ≥ 2xULN or</td></tr>
</table>

Liver Chemistry Stopping Criteria	
• Repeat liver chemistries (include ALT, AST, alkaline phosphatase, bilirubin) and perform liver event follow up assessments within **24-72 hrs** • Monitor subjects weekly until liver chemistries resolve, stabilize or return to within baseline	**INR >1.5:** • Anti-nuclear antibody, anti-smooth muscle antibody, Type 1 anti-liver kidney microsomal antibodies, and quantitative total immunoglobulin G (IgG) or gamma globulins. • Serum acetaminophen adduct high performance liquid chromatography (HPLC) assay (quantifies potential acetaminophen contribution to liver injury in subjects with definite or likely acetaminophen use in the preceding week). • Liver imaging (ultrasound, magnetic resonance, or computerised tomography) and /or liver biopsy to evaluate liver disease; complete Liver Imaging and/or Liver Biopsy CRF forms.

1. Serum bilirubin fractionation should be performed if testing is available. If serum bilirubin fractionation is not immediately available, discontinue study treatment for that subject if ALT ≥ 3xULN and bilirubin ≥ 2xULN. Additionally, if serum bilirubin fractionation testing is unavailable, record presence of detectable urinary bilirubin on dipstick, indicating direct bilirubin elevations and suggesting liver injury.
2. All events of ALT ≥ 3xULN and bilirubin ≥ 2xULN (>35% direct bilirubin) or ALT ≥ 3xULN and INR>1.5, if INR measured, which may indicate severe liver injury (possible 'Hy's Law'), must be reported as an SAE (excluding studies of hepatic impairment or cirrhosis); INR measurement is not required and the threshold value stated will not apply to subjects receiving anticoagulants
3. Includes: Hepatitis A IgM antibody; Hepatitis B surface antigen and Hepatitis B Core Antibody (IgM); Hepatitis C RNA; Cytomegalovirus IgM antibody; Epstein-Barr viral capsid antigen IgM antibody (or if unavailable, obtain heterophile antibody or monospot testing); Hepatitis E IgM antibody
4. PK sample may not be required for subjects known to be receiving placebo or non-GSK comparator treatments. Record the date/time of the PK blood sample draw and the date/time of the last dose of study treatment prior to PK blood sample draw on the CRF. If the date or time of the last dose is unclear, provide the subject's best approximation. If the date/time of the last dose cannot be approximated OR a PK sample cannot be collected in the time period indicated above, do not obtain a PK sample. Instructions for sample handling and shipping are in the SRM.