Statistical Analysis Plan

Study ID: 213585

Official Title of Study: A Randomized, Placebo Controlled, Double Blind, Single and Repeat Dose Escalation Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of GSK3915393 in Healthy Participants and open label assessment of coadministration of GSK3915393 with grapefruit juice and itraconazole on the pharmacokinetics of GSK3915393

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TITLE PAGE

Protocol Title: A Randomized, Placebo Controlled, Double Blind, Single and Repeat Dose Escalation Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of GSK3915393 in Healthy Participants and open label assessment of coadministration of GSK3915393 with grapefruit juice and itraconazole on the pharmacokinetics of GSK3915393

Protocol Number: 213585 / Amendment 02

Compound Number: GSK3915393

Short Title: A Phase 1 Study to Evaluate the Safety, Tolerability, and Pharmacokinetics of GSK3915393 in Healthy Participants and to evaluate the interaction between GSK3915393 and grapefruit juice and itraconazole.

Sponsor Name: GlaxoSmithKline Research & Development Limited

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TABLE OF CONTENTS

PAGE

1. INTRODUCTION 6
1.1. Objectives, Endpoints and Estimands 6
1.1.1. Objectives and Endpoints 6
1.1.2. Estimands 9
1.2. Study Design 14

2. STATISTICAL HYPOTHESES 18

3. ANALYSIS SETS 19

4. STATISTICAL ANALYSES 19
4.1. General Considerations 19
4.1.1. General Methodology 19
4.1.2. Baseline Definition 20
4.2. Safety Analyses 20
4.2.1. Adverse Events Analyses 20
4.2.2. Clinical Laboratory Analyses 21
4.2.3. Other Safety Analyses 21
4.3. Pharmacokinetic Analyses 21
4.3.1. Pharmacokinetic Parameters 21
4.3.2. Single Dose Pharmacokinetics (part A) 24
4.3.2.1. Endpoints/Variables 24
4.3.2.2. Main Analytical Approach 24
4.3.3. Repeat Dose Pharmacokinetics (part B) 24
4.3.3.1. Endpoints/Variables 24
4.3.3.2. Main Analytical Approach 25
4.3.4. Assessment of Food Effect (Part B) 26
4.3.4.1. Endpoints/Variables 26
4.3.4.2. Main Analytical Approach 26
4.3.5. CYP3A4 victim assessment – Impact of Grapefruit Juice (Part C) 27
4.3.5.1. Endpoints/Variables 27
4.3.5.2. Main Analytical Approach 27
4.3.6. CYP3A4 victim assessment – impact of itraconazole (part C) 27
4.3.6.1. Endpoints/Variables 27
4.3.6.2. Main Analytical Approach 27
4.3.7. Additional Part C Assessments 28
4.3.7.1. Endpoints/Variables 28
4.3.7.2. Summary Measure 28
4.3.8. Other Endpoints 28
4.3.8.1. Endpoints/Variables 28
4.3.8.2. Summary Measure 28
4.4. Interim Analyses 28
4.5. Changes to Protocol Defined Analyses 28

5. SAMPLE SIZE DETERMINATION 28

6. SUPPORTING DOCUMENTATION 30
6.1. Appendix 1 Abbreviations and Trademarks 30

6.1.1. List of Abbreviations 30
6.1.2. Trademarks 31

7. REFERENCES 32

Version history

Table 1 SAP Version History Summary

SAP Version	Document Date	Protocol Version (Date) on which SAP is Based	Change	Rationale
1	22 OCT 2020	1.0	Not Applicable	Original version
2	24 MAR 2021	2.0	Revised Part C study design and related sections and minor modifications in part B	Updated to align with Part C study design change and other minor changes covered in protocol amendment 2 and "213585 File Note_Changes to feeding regimen and add additional pre dose PK samples in Part B Cohort 2"

1. INTRODUCTION

The purpose of this SAP is to describe the planned analyses for study parts A, B and C to be included in the Clinical Study Report for Protocol 213585. All details of the dose escalation process and associated data review is outlined in the dose escalation plan. Metabolite evaluations will be conducted by DMPK and is not covered in this SAP.

Descriptive study population analyses such as summary of demography and baseline characteristics and additional detail with regards to data handling conventions and the specification of data displays will be provided in the Output and Programming Specification (OPS) document.

1.1. Objectives, Endpoints and Estimands

1.1.1. Objectives and Endpoints

Objectives	Endpoints
Primary Objectives	**Primary Endpoints**
Part A HV Dose Escalation Single Dose • To evaluate the safety and tolerability of single escalating oral doses of GSK3915393 administered in the fed state in healthy adult participants **Part B HV Dose Escalation Repeat Dose** • To evaluate the safety and tolerability of repeat escalating oral doses of GSK3915393 twice daily (BID) administered in the fed and fasted state in healthy adult participants **Part C CYP3A4 Victim Interaction** • To characterize the PK profile of IV GSK3915393 administered alone and when co-administered with itraconazole in healthy adult participants • To characterize the PK profile of oral GSK3915393 administered	**Part A and B** • Occurrence of serious adverse events (SAEs), adverse events (AEs) and treatment related AEs • Occurrence of clinically significant changes in physical examination, vital signs, laboratory parameters, and 12-lead electrocardiogram (ECG) findings. **Part C** • Cmax, tmax, AUC(0-t), AUC (0-∞) and ($t_{1/2}$) as appropriate

Objectives	Endpoints
alone and when co-administered with grapefruit juice or with itraconazole in healthy adult participants	
Secondary Objectives	**Secondary Endpoints**
Part A HV Dose Escalation Single Dose • To characterize the PK profile of single oral doses of GSK3915393 in healthy adult participants • To characterize the PK profile of a single intravenous dose of GSK3915393 in healthy adult participants • To evaluate the safety and tolerability of single microdose doses of GSK3915393 in healthy adult participants **Part B HV Dose Escalation Repeat Dose** • To characterize the PK profile of single doses and repeat oral doses of GSK3915393 in healthy adult participants	**Part A** • Maximum observed plasma drug concentration (Cmax), time to maximum observed plasma drug concentration (tmax), area under the plasma drug concentration versus time curve from time zero to last quantifiable concentration (AUC(0-t)), AUC from time zero to infinity (AUC(0-∞)), and apparent terminal half-life ($t_{1/2}$) as appropriate. • Clearance (CL) and volume of distribution (Vd) following IV administration and absolute bioavailability (F) of oral administration • Fraction of drug escaping hepatic metabolism (F_H) and product of fraction of drug absorbed (F_A) and fraction of drug escaping gut metabolism (F_G) i.e., (F_A*F_G). Clinical safety and tolerability data including adverse events (AEs) and serious adverse events (SAEs), clinically significant changes in laboratory values, vital signs and 12 lead electrocardiogram (ECG) readings and physical examinations **Part B** • Cmax, tmax, AUC(0-t), and AUC over the dosing interval AUC (0-τ)

Objectives	Endpoints
• To assess the impact of food on the PK of GSK3915393 in healthy adult participants • To evaluate time to steady-state **Part C CYP3A4 Victim Interaction** • To evaluate the safety and tolerability of a single oral dose of GSK3915393 when administered with grapefruit juice or with itraconazole in healthy adult participants • To evaluate the safety and tolerability of single intravenous dose of GSK3915393 when administered with itraconazole in healthy adult participants • To investigate absorption characteristics and first pass clearance of GSK3915393	• Cmax, tmax and AUC (0-τ) following 1st dose of day • Pre-dose concentrations on Days 2, 3, 5, 7 and 14 **Part C** • Occurrence of serious adverse events (SAEs), adverse events (AEs) and treatment related AEs • Occurrence of clinically significant changes in physical examination, vital signs, laboratory parameters, and 12-lead electrocardiogram (ECG) findings. • F_A, F_G and F_H
Exploratory Objectives	**Exploratory Endpoints**
• To investigate metabolites of GSK3915393 in plasma and urine following oral dosing (Part A and B) • To investigate metabolites of GSK3915393 in duodenal bile following oral dosing (Part B, highest dose only) • To assess potential effect of repeat doses of GSK3915393 on Cytochrome P450 3A4 (CYP3A4) enzyme activity (Part B). • To assess the effect of single dose GSK3915393 on Holter ECG in healthy volunteers. Waveforms will be stored for potential future analysis.	• Metabolites of GSK3915393 in plasma and urine. The analyses will be conducted and reported separately. • Metabolites of GSK3915393 in duodenal bile. The analyses will be conducted and reported separately. • Plasma 4β-hydroxycholesterol to cholesterol ratio at pre-treatment and following repeat dosing of GSK3915393 • If analyses conducted: Centrally read ECG parameters and correlation between plasma levels of GSK3915393 and QTc changes. These analyses if conducted will be detailed in a separate analysis plan.

1.1.2. Estimands

In Table 2, the possible intercurrent events and the rationale for relating them to treatment or otherwise is described. Individual participant data will be included in data listings regardless of the strategies followed for the analyses.

Table 2 Estimands

The following two attributes apply to all estimands:

- Treatment condition: GSK3915393 vs Placebo
- Population: Healthy Volunteers.

Estimand Category	Estimand		
	Variable/ Endpoint	**Intercurrent Event Strategy**	**Population Level Summary Measure**
Primary Objective (1): To evaluate the safety and tolerability of single escalating oral doses of GSK3915393 administered in the fed state in healthy adult participants.			
Primary 1 (Part A)	• Adverse events • Clinically significant changes from baseline in laboratory data • Clinically significant changes from baseline in vital signs • Clinically significant changes from baseline in 12 lead electrocardiograms (ECGs) • Clinically significant changes from baseline in physical examination	• **All Intercurrent Events** - All data will be reported as captured • (**Treatment policy strategy**, where we are interested in evaluating all adverse events including clinically significant other safety findings occurring after the start of treatment)	Descriptive Statistics

Estimand Category	Estimand		
	Variable/ Endpoint	**Intercurrent Event Strategy**	**Population Level Summary Measure**
Primary Objective (2): To evaluate the safety and tolerability of repeat escalating oral doses of GSK3915393 twice daily (BID) administered in the fed and fasted state in healthy adult participants.			
Primary 2 (Part B)	As defined for Primary Objective 1	As Defined for Primary Objective 1	As Defined Primary for Objective 1
Primary Objective (3): To characterize the PK profile of IV GSK3915393 administered alone and when co-administered with itraconazole in healthy adult participants			
Primary 3 (Part C)	• Cmax, • tmax, • AUC(0-t), • AUC(0-∞) and • $t_{1/2}$	• **All Intercurrent Events** – a treatment policy approach will be applied such that all available data will be included in analyses. For subjects with partial concentration-time data on a given study period, derived parameters such as AUC will be available on that study period provided there are sufficient concentration data to enable their estimation	Descriptive statistics Point estimates and 90% CI for ratios of AUC or Cmax for GSK3915393 IV with ITZ vs GSK3915393 IV.
Primary Objective (4): To characterize the PK profile of oral GSK3915393 administered alone and when co-administered with grapefruit juice or with itraconazole in healthy adult participants			
Primary 4 (Part C)	As defined for Primary Objective 3	As Defined for Primary Objective 3	As Defined Primary for Objective 3 Point estimates and 90% CI for ratios of AUC or Cmax for GSK3915393 PO with GFJ vs GSK3915393 PO with water and GSK3915393 PO with ITZ vs GSK3915393 PO with water

Estimand Category	Estimand		
	Variable/ Endpoint	**Intercurrent Event Strategy**	**Population Level Summary Measure**
Secondary Objective (1): To characterize the PK profile of single oral doses of GSK3915393 in healthy adult participants			
Secondary 1 (Part A)	• AUC (0-∞) • AUC (0-t) • Cmax • tmax • $t_{1/2}$ • Absolute bioavailability (F) • F_H • F_A*F_G	As Defined for Primary Objective 3	Descriptive Statistics
Secondary Objective (2): To characterize the PK profile of single intravenous doses of GSK3915393 in healthy adult participants			
Secondary 2 (Part A)	• AUC (0-∞) • AUC (0-t) • Cmax • Tmax • $t_{1/2}$ • Clearance (CL) (IV only) • Volume of distribution (Vd) (IV only)	As Defined for Primary Objective 3	Descriptive Statistics
Secondary Objective (3): To evaluate the safety and tolerability of single microdose doses of GSK3915393 in healthy adult participants.			
Secondary 3 (Part A)	As defined for Primary Objective 1	As defined for Primary Objective 1	As defined for Primary Objective 1
Secondary Objective (4): To characterize the PK profile of single doses and repeat oral doses of GSK3915393 in healthy adult participants.			
Secondary 4 (Part B)	• AUC (0-24) • AUC over the dosing interval AUC (0-τ) • Cmax after 1st and 2nd dose	As defined for Primary Objective 3	Descriptive Statistics Geometric mean & 90% CI for accumulation ratios

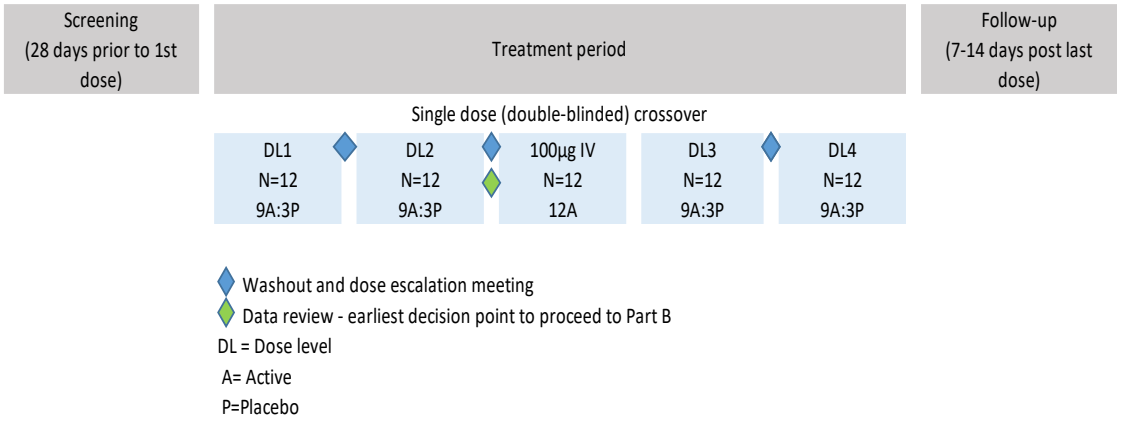
Estimand Category	Estimand		
	Variable/ Endpoint	**Intercurrent Event Strategy**	**Population Level Summary Measure**
	• tmax after 1st and 2nd dose • Trough concentrations C_τ • $t_{1/2}$ after 1st dose on day 1 • Accumulation ratios (Cmax accumulation, Ro)		
Secondary Objective (5): To access the impact of food on the PK of GSK3915393 in healthy adult participants			
Secondary 5 (Part B)	• AUC over the dosing interval AUC (0-τ) after 1st dose • Cmax after 1st dose • tmax after 1st dose	As defined for Primary Objective 3	Descriptive statistics Geometric mean ratio and 90% CI for AUC and Cmax Median difference and 90% CI for tmax
Secondary Objective (6): To evaluate time to steady-state			
Secondary 6 (Part B)	• Pre-dose concentrations on Days 1-14 (days 2, 3, 5, 7 and 14 only in cohort 1)	As defined for Primary Objective 3	Descriptive statistics Point estimate and 90% CI for day effect
Secondary Objective (7): To evaluate the safety and tolerability of a single oral dose of GSK3915393 when administered with grapefruit juice or with itraconazole in healthy adult participants			
Secondary 7 (Part C)	As defined for Primary Objective 1	As defined for Primary Objective 1	As defined for Primary Objective 1
Secondary Objective (8): To evaluate the safety and tolerability of single intravenous dose of GSK3915393 when administered with itraconazole in healthy adult participants			
Secondary 8 (Part C)	As defined for Primary Objective 1	As defined for Primary Objective 1	As defined for Primary Objective 1
Secondary Objective (9): To investigate absorption characteristics and first pass clearance of GSK3915393			

Estimand Category	Estimand		
	Variable/ Endpoint	**Intercurrent Event Strategy**	**Population Level Summary Measure**
Secondary 9 (Part C)	• F • F_A F_G and F_H	As defined for Primary Objective 3	Descriptive statistics

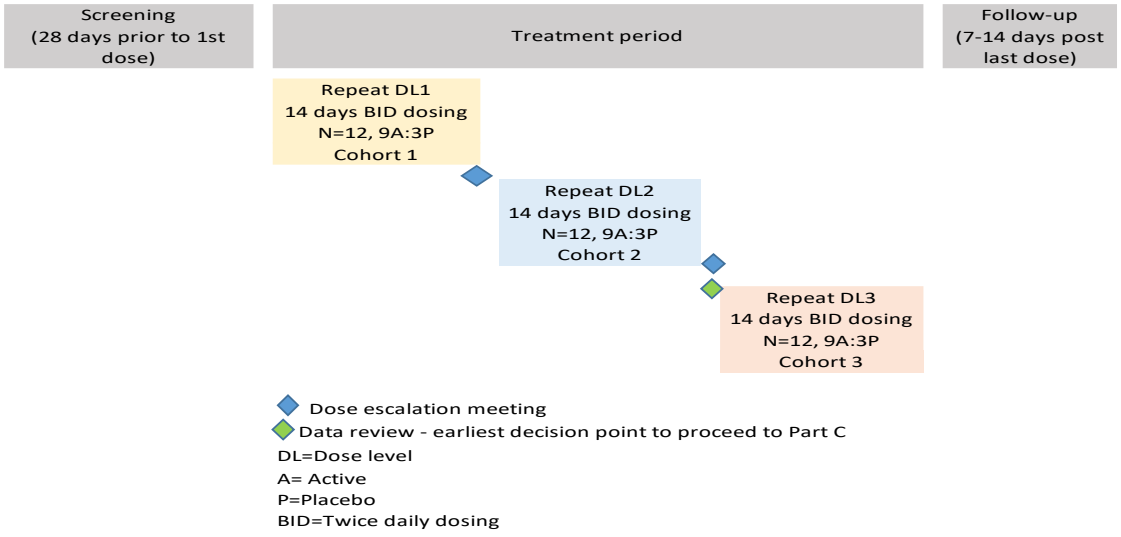
1.2. Study Design

Overview of Study Design and Key Features

Part A – Single Dose

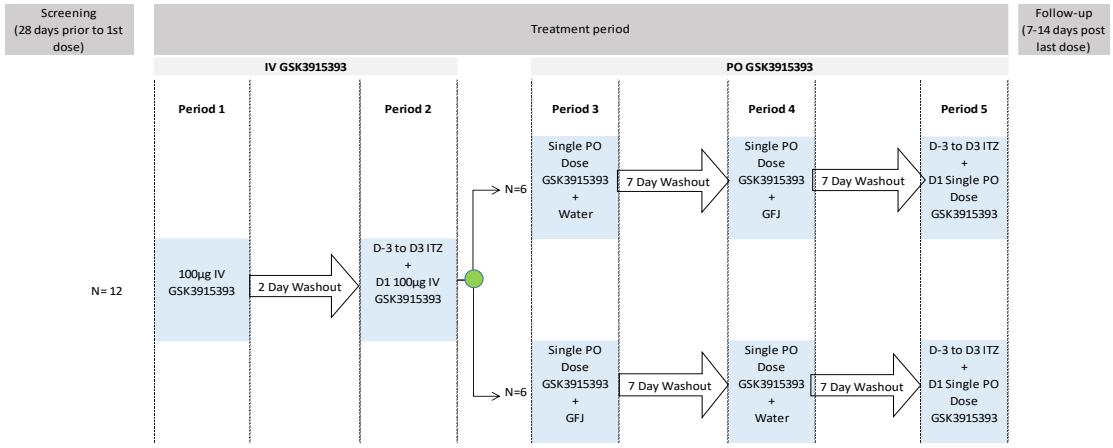


Part B – Repeat Dose



Overview of Study Design and Key Features

Part C – CYP3A4 Vicitim Interaction



D = Day
GFJ = Grapefruit Juice
ITZ = Itraconazole
N = Number of participants
washout of at least 7 days and IV data review to select PO dose.
NOTE: Washout periods represent the minimum duration required between each period and may be longer for operational purposes.

Design Features	This FTIH study will be a randomized, double-blind, placebo controlled, single center trial in two parts. **Part A** is a crossover design, single dose (SD), dose escalation study in one cohort of healthy participants. Participants will receive single ascending doses of GSK3915393 or matching placebo as an oral dose in periods 1, 2, 4 and 5 and as a single IV microdose dose in period 3. The initial dosing for all periods in which the dose level has been escalated will be staggered so that 2 participants will be dosed as sentinel participants, one with study drug and one with placebo. After approximately 24 hours, and provided the investigator considers the safety and tolerability of the sentinels to be acceptable, the remainder of the participants scheduled for the period may be dosed. Sentinel dosing is not required in the IV dosing period. **Part B** is a parallel group,14-day, repeat oral dose, dose escalation study in 3 cohorts of healthy participants. Participants will receive GSK3915393 or matching placebo BID in each of the sequential cohorts. The initial dosing for all periods in which the dose level has been escalated will be staggered so that 2 participants will be dosed as sentinel participants, one with study drug and one with placebo. After at least 4 days of dosing, and provided the investigator considers the safety and tolerability of the sentinels to be acceptable, the remainder of the participants scheduled for the period may be dosed. The impact of food on the PK of GSK3915393 will be investigated following the

Overview of Study Design and Key Features	
	AM dose on three days in all cohort (day 3=fasted, day 5= high fat breakfast, day 7=standard breakfast). The participants, investigator, and study staff are all blinded. **Part C** is a 5 Single Dose periods in healthy adult participants. In period 1 all participants will receive an IV microdose of GSK3915393. In period 2, all participants will receive an IV microdose of GSK3915393 following prior dosing with itraconazole. In periods 3 and 4, participants will receive oral GSK3915393 with or without grapefruit juice in a randomized crossover design. In period 5, all participants will receive oral GSK3915393 following prior dosing with itraconazole.
Study Intervention	**Part A** – Participants will receive GSK3915393 extemporaneous formulations: API filled capsules and placebo capsules according to the treatment sequence to which they are assigned. For the IV dose, participants will receive GSK3915393 at a dose of 100 µg within 24 hours from compounding. **Part B** – Participants will receive GSK3915393 extemporaneous formulations: API filled capsules and placebo capsules according to the treatment to which they are assigned. **Part C** – The IV microdose of GSK3915393 will be 100 µg. The oral dose of GSK3915393 to be given in periods 3-5 will be selected after review of the IV PK data from periods 1 and 2 and will not exceed 20 mg. Participants would be either dosed GSK3915393 alone, with grapefruit juice or with itraconazole. Participants will be dosed in the fasted state.
Study Intervention Assignment	Part A Cohort 1 participants will be randomized to one of the 4 treatment sequences and will receive 1 oral placebo dose and 4 active doses (3 as an oral GSK3915393 dose and 1 as a single GSK3915393 IV microdose): In each cohort in Part B, 9 participants on active versus 3 participants in placebo (i.e., in a 3:1 ratio) In Part C, twelve participants will be randomized to one of the 2 sequences and will receive 2 IV microdose of GSK3915393 and 3

Overview of Study Design and Key Features	
	active oral doses of GSK3915393 either alone, with grapefruit juice or with itraconazole.
Interim Analysis	The decision to proceed to higher dose strengths in parts A and B will be made by the Data Escalation Committee (DEC) based on assessment of safety, tolerability and pharmacokinetic data at the preceding doses. In part C, an interim evaluation of the pharmacokinetic and safety data in periods 1 and 2 (IV GSK3915393 + ITZ or water) in conjunction with available safety data from parts A and B will be conducted by the DEC to support the selection of the GSK3915393 oral dose to be administered in periods 3-5. The DEC will review blinded outputs which may include individual participant data, tabular summaries, graphical presentations and statistical analysis. GSK members of the DEC may additionally review unblinded data in a GSK only closed session of the DEC. Unblinded data will not be shared with the investigators/site who will remain blinded throughout. Further details are provided in the dose escalation plan.

2. STATISTICAL HYPOTHESES

The primary objectives of parts A and B of this study are to assess the safety and tolerability of single and repeat ascending doses of GSK3915393 in healthy participants and the primary objective of part C is to evaluate the pharmacokinetic interaction between GSK393153 and grapefruit juice and itraconazole.

No formal hypotheses will be tested. However, wherever appropriate, an estimation approach has been used, and point estimates and confidence intervals (CIs) will be constructed.

3. ANALYSIS SETS

For the purposes of analysis, the following analysis sets are defined.

Analysis Set	Definition / Criteria	Analyses Evaluated
Screened	• All participants who were screened for eligibility.	• Study Population
Randomised	• All participants who were randomly assigned to treatment in the study. • This population will be based on the treatment the participant was randomized to.	• Study Population
Safety	• All randomized participants who received at least one dose of study treatment. • This population will be based on the treatment the participant actually received.	• Safety • Study Population
COVID-19	• All participants in the Safety set who had a confirmed, probably or suspected COVID-19 case diagnosis.	• Study Population • Safety
Pharmacokinetic (PK)	• All participants in the Safety population who had at least 1 non-missing PK assessment. (Non-quantifiable [NQ] values will be considered as non-missing values). • This population will be based on the treatment the participant actually received.	• PK

4. STATISTICAL ANALYSES

4.1. General Considerations

The study population analyses will be based on the Safety set, unless otherwise specified. Safety analyses will also be based on the safety set and PK analyses will be based on the PK set.

4.1.1. General Methodology

Safety evaluations are planned to be descriptive in nature. Statistical modelling will be performed for Pharmacokinetic data, and point estimates with 90% CIs will be constructed.

Unless otherwise specified, summary tables will provide the following descriptive statistics as a minimum:

- Continuous data:
  n, mean, standard deviation (SD), median, minimum and maximum.
- Log transformed PK parameters:
  n, geometric mean, 95% confidence interval for geometric mean, SD on the log scale (SD (log)), Between subject coefficient of variation (%CVb).
- Categorical data:
  number and percentage of participants in each category.

4.1.2. Baseline Definition

Baseline is defined as the last non-missing assessment prior to first dose.

For some ECG and Vital Signs parameters, the pre-dose data is captured as triplicate. In these cases, the baseline will be defined as the mean of the triplicate assessments.

For Part A and B and periods 1, 3 and 4 in part C, the day 1 pre-dosing assessment will be the baseline. However, if there are no pre-dose assessment collected at Day 1, the last available data from either Day -1 or screening (as schedule of assessment in each Cohort permits) will be defined as baseline. If there are no valid assessments from day 1, day -1 or screening, the baseline will be set to missing.

In part A & C, period baselines will be defined utilising the assessments taken in the specific period. Participant level baselines will also be computed and will be the means of the available period level baselines for each participant.

For Part C in periods 2 and 5, the day 1 pre dosing assessment (prior to the day 1 dose of both ITZ and GSK39315393) will be the baseline. However, if this assessment is missing, data collected from days -1 to day -3 will be considered. The closest assessment prior to day 1 pre dose will be set as the baseline. If there are no valid assessments, the baseline will be set to missing.

4.2. Safety Analyses

The primary objective of this study is to evaluate the safety and tolerability of GSK3915393. Safety data will be summarized by dose regimen over time for the safety population set.

4.2.1. Adverse Events Analyses

Adverse events analyses including summaries AEs, SAEs, treatment related AEs and other significant AEs will be based on GSK Core Data Standards.

COVID-19 events will be summarized and listed based on GSK Core Data Standards.

Further details will be provided in the Output Programming Specifications (OPS).

4.2.2. Clinical Laboratory Analyses

Laboratory evaluations including summaries of Chemistry laboratory tests, Haematology laboratory tests, Urinalysis, and Liver function tests will be based on GSK Core Data Standards.

Further details and the potentially clinical important values will be provided in the OPS.

4.2.3. Other Safety Analyses

The summaries of non-laboratory safety test results including vital signs, 12 lead ECG, telemetry, Physical Examination and Pregnancy will be based on GSK Core Data Standards, unless otherwise specified. Further details and the potentially clinical important values will be provided in the OPS.

4.3. Pharmacokinetic Analyses

Part A and B PK analyses planned for the study are a part of the secondary objectives and Part C PK analyses planned for the study is a part of the primary objectives and will be based on the PK set. Data displays will be based on GSK Data Standards and statistical principles. Full details of the planned displays are provided in the OPS.

4.3.1. Pharmacokinetic Parameters

Pharmacokinetic parameters defined in Table 3 will be determined from the GSK3915393 plasma concentration-time data, as data permits. Pharmacokinetic parameters will be calculated using standard non-compartmental analysis and using WinNonlin version 8.0 (or higher).

All calculations of non-compartmental parameters will be based on actual sampling times. For dose escalation meetings nominal sampling times will be used.

Additionally, Itraconazole plasma concentration data collected during Period 2 and 5 in Part C would be summarized separately.

Table 3 Pharmacokinetic Parameters

Parameter	Parameter Description
Part A	
AUC (0-∞)	Area under the concentration-time curve extrapolated to infinity will be calculated as: $$AUC(0-\infty) = AUC(0-t) + \frac{C(t)}{\lambda_z}$$ (NOTE: λ_z is the terminal phase rate constant and C(t) is the last quantifiable concentration. % of the extrapolated area should not exceed 20%. Any value of AUC (0-∞) derived with more than 20% extrapolation should be flagged and excluded from summary statistics if >30%).
AUC(0-t)	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration (C(t)) will be calculated using the linear trapezoidal rule for each incremental trapezoid and the loge trapezoidal rule for each decremental trapezoid.
Cmax	Maximum observed concentration, determined directly from the concentration-time data.
tmax	Time to reach Cmax, determined directly from the concentration-time data.
$t_{1/2}$	Apparent terminal half-life will be calculated as: $$t_{1/2} = \frac{ln\ 2}{\lambda_z}$$
CL	The clearance will be calculated as Dose/AUC (0-∞). Calculated only for IV dose regimen.
V_d	Volume of distribution for IV dose regimen.
F	Absolute bioavailability calculated as [$AUC(0\text{-}\infty)_{po}$/$dose_{po}$] / [$AUC\ (0\text{-}\infty)_{iv}$ /$dose_{iv}$]
F_H	Fraction of drug escaping hepatic metabolism (F_H) is calculated as $F_H = (1\text{-}E_H)$ (E_H = Hepatic extraction ratio) • Where, $E_H = CL_{H,b} \div Q_{H,b}$ • $CL_{H,b}$= hepatic blood clearance; $Q_{H,b}$ = hepatic blood flow (1.26 L/h/kg) • $CL_{H,b}$ (Dose IV ÷ Plasma AUC(0-inf)) IV ÷ 0.64 (Blood : Plasma)
F_A*F_G	Fraction of drug absorbed intact * Fraction of drug escaping gut metabolism (F_A*F_G) is calculated as (F_A*F_G) = F÷F_H

Parameter	Parameter Description
Part B	
AUC (0-10)	Area under the concentration-time dosing interval curve following 1st dose from time zero to 10 hours post-dose
AUC (10-24)	Area under the concentration-time dosing interval curve following 2nd dose from 10 hours to 24 hours post-dose.
AUC (0-24)	Area under the concentration-time curve from time zero to 24 hours post-dose.
Cmax 1st dose	Maximum observed concentration following 1st dose (0-10h), determined directly from the concentration-time data.
Cmax 2nd dose	Maximum observed concentration following 2nd dose (10-24h), determined directly from the concentration-time data.
tmax 1st dose	Time to reach Cmax following 1st dose (0-10h), determined directly from the concentration-time data.
tmax 2nd dose	Time to reach Cmax following 2nd dose (10-24h), determined directly from the concentration-time data.
Ctrough	Pre-dose concentrations on days 1-14 (days 2 3,5,7 and 14 in cohort 1*)
$t_{1/2}$	Apparent terminal half-life will be calculated as: $$t_{1/2} = \frac{ln\ 2}{\lambda_z}$$
Cmax accumulation	Cmax accumulation will be calculated as Cmax 1st $Dose_{D7}$/Cmax 1st $Dose_{D1}$
Ro	Observed accumulations ratio will be calculated as $AUC\ (0\text{-}10)_{D7}$ / $AUC(0\text{-}10)_{D1}$
Part C (CYP3A4 victim assessment)	
Cmax	Maximum observed concentration, determined directly from the concentration-time data.
tmax	Time to reach Cmax, determined directly from the concentration-time data.
AUC(0-t)	Area under the concentration-time curve from time zero to the time of the last quantifiable concentration (C(t)) will be calculated using the linear trapezoidal rule for each incremental trapezoid and the loge trapezoidal rule for each decremental trapezoid.
AUC (0-∞)	Area under the concentration-time curve extrapolated to infinity will be calculated as: $$AUC(0-\infty) = AUC(0-t) + \frac{C(t)}{\lambda_z}$$
$t_{1/2}$	Apparent terminal half-life will be calculated as:

Parameter	Parameter Description
	$t_{1/2} = \frac{ln\ 2}{\lambda_z}$
F_G[1]	$F_G = AUC_{H2O} \div AUC_{GFJ}$ (oral administration of GSK3915393)
F	Absolute bioavailability calculated as $[AUC(0\text{-}\infty)_{po}/dose_{po}]$ / $[AUC\ (0\text{-}\infty)_{iv}\ /dose_{iv}]$
F_H	Fraction of drug escaping hepatic metabolism (F_H) is calculated as $F_H = (1\text{-}E_H)$ (E_H = Hepatic extraction ratio) • Where, $E_H = CL_{H,b} \div Q_{H,b}$ • $CL_{H,b}$= hepatic blood clearance; $Q_{H,b}$ = hepatic blood flow (1.26 L/h/kg) $CL_{H,b}$ (Dose IV ÷ Plasma AUC(0-inf)) IV ÷ 0.64 (Blood : Plasma)
F_A	$F_A = F \div (F_H * F_G)$
CL	The clearance will be calculated as Dose/AUC (0-∞). Calculated only for IV dose regimen.

[1] Calculation of F_G assumes that metabolism of GSK3915393 in gut is solely mediated by CYP3A4 and that GFJ inhibits gut but not hepatic CYP3A4. Calculation uses AUC (0-∞)
[or AUC (0-t) or AUC (0-τ) where AUC (0-∞) cannot be derived].

4.3.2. Single Dose Pharmacokinetics (part A)

4.3.2.1. Endpoints/Variables

AUC (0-∞), AUC (0-t), Cmax, tmax, $t_{1/2}$, F following single oral doses of GSK3915393.

AUC (0-∞), AUC (0-t), Cmax, tmax, $t_{1/2}$, CL, V_d following IV dose of GSK3915393.

4.3.2.2. Main Analytical Approach

All pharmacokinetic parameters will be summarised. Full details will be provided in the OPS. Dose normalised AUC (0-∞) [or AUC(0-t) if AUC (0-∞) cannot be derived] and Cmax will be displayed graphically versus dose to provide a visual assessment of dose linearity.

4.3.3. Repeat Dose Pharmacokinetics (part B)

4.3.3.1. Endpoints/Variables

Assessment of the pharmacokinetics upon repeat dosing will be based on AUC(0-τ), Cmax and tmax following 1st and 2nd dose, trough concentrations, $t_{1/2}$ (day 1 only, if data permit), Cmax accumulation ratio and Ro.

In addition to the PK sampling schedule outlined in the protocol a pre dose sample would be collected pre dose on days 4, 6, 8, 9, 10, 11, 12 and 13 in cohorts 2 and 3 of part B.

4.3.3.2. Main Analytical Approach

a. All pharmacokinetic parameters will be summarised by time and dose. Full details will be provided in the OPS.

b. Dose normalised AUC(0-τ) and Cmax after 1st and 2nd doses will be assessed descriptively.

c. Log_e transformed AUC (0- τ) for the first dose on Days 1 and 7 will be analysed by a mixed effect model with dose, day (Day 1 and 7) and dose-by-day interaction fitted as fixed effects and participant as a random effect. The Kenward & Roger (KR) degrees of freedom approach will be used. The difference between the least square means for day 7 and day 1 at each dose and associated 90% CI will be exponentially back transformed to obtain point and 90% CI estimates for Ro for each active dose. Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values.

d. Log_e transformed Cmax for the first dose on Days 1 and 7 will be analysed by a mixed effect model with dose, day (Day 1 and 7) and dose-by-day interaction fitted as fixed effects and participant as a random effect. The Kenward & Roger (KR) degrees of freedom approach will be used. The difference between the least square means for the day 7 and day 1 at each dose and associated 90% CI will be exponentially back transformed to obtain point and 90% CI estimates for Cmax accumulation ratio for each active dose. Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values.

e. A preliminary assessment of time to achieve steady state will be made based on visual inspection, of pre dose concentrations plotted by day for each dose in order to determine the time points (days) to be included in the statistical analysis described in f.

f. A mixed effects model will be fitted to log_e transformed pre dose concentrations with day (continuous variable) as fixed effects and participant as a random effect for each dose separately. The KR degrees of freedom approach will be used. The coefficient for the slope of the day effect on the loge-scale will be used to evaluate steady state for each dose. The 90% CIs for the slope for each dose will be calculated. Estimates should be exponentially back transformed to be presented. A slope of 1 indicates steady state. If individual dose levels are inconclusive on steady state, combined dose analysis would be considered. Distributional assumptions underlying the model used

for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values.

4.3.4. Assessment of Food Effect (Part B)

4.3.4.1. Endpoints/Variables

Food effect assessments will be based on AUC (0-τ), Cmax and tmax after 1st dose.

4.3.4.2. Main Analytical Approach

For each dose level separately, Log_e-transformed AUC(0-τ) and Cmax after 1st dose (on days 3, 5 and 7) will be statistically analysed using a Mixed effects model with:

- Prandial state ~ (high fat (day 5 data), standard meal (day 7 data) or fasted (day 3 data))
- participant as random effect

The Kenward and Roger method for approximating the denominator degrees of freedom and correcting for bias in the estimated variance-covariance of the fixed effects will be used. An unstructured type of the covariance matrices R (accounting for the within participant variability) and G (accounting for the between participant variability) will be used.

The estimated geometric mean ratios and 90% CI for (high fat meal: fasted) and (standard meal: fasted) will be derived for each dose (i.e., Low dose, Medium dose and High dose).

Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values (i.e. checking the normality assumption and constant variance assumption of the model respectively) to gain confidence that the model assumptions are reasonable. If there are any departures from the distributional assumptions, alternative transformations, such as data squared or square root of data, will be explored. Non-parametric analyses will be conducted if the normality assumption does not hold for any of the alternative transformations. If this model fails to converge, alternative covariance structures may be considered in the following order (1) Compound Symmetry (CS) and (2) Variance Components (VC).

tmax after 1st dose on day 3 and day 5 will be analysed non parametrically applying a Wilcoxon signed rank test and a point estimate and distribution free 90% CI for the median difference in tmax for (high fat meal vs fasted) will be derived by specifying the CIQUANTDF option in SAS Proc Univariate. The analysis will be repeated for tmax after 1st dose on days 3 and day 7 to derive point estimate and distribution free 90% CI for the median difference of (standard meal vs fasted).

4.3.5. CYP3A4 victim assessment – Impact of Grapefruit Juice (Part C)

4.3.5.1. Endpoints/Variables

AUC (0-∞) [or AUC (0-t) or AUC (0-τ)], Cmax following single oral doses of GSK3915393 with water or with GFJ.

4.3.5.2. Main Analytical Approach

Log transformed AUC (0-∞) [or if not available AUC(0-t) or AUC (0-τ)] for periods 3 and 4 will be analysed utilising a mixed model with fixed regimen and period effects and random subject effect. Between regimen differences will be computed and back transformed to provide point estimate and 90% CI for the ratio of AUC for GSK3915393 PO with GFJ: GSK3915393 PO + water, and an estimate of F_G defined as the ratio of AUC GSK3915393 PO with water: GSK3915393 PO + GFJ. Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values.

Similar analysis will be conducted for log transformed Cmax to provide an estimate of the ratio of Cmax for GSK3915393 PO with GFJ: GSK3915393 PO + water.

In addition, descriptive summaries of AUC and Cmax will be produced for each regimen.

4.3.6. CYP3A4 victim assessment – impact of itraconazole (part C)

4.3.6.1. Endpoints/Variables

AUC (0-∞) [or AUC (0-t) or AUC (0-τ)], Cmax following single IV / oral doses of GSK3915393 alone/with water or with ITZ.

4.3.6.2. Main Analytical Approach

Log transformed AUC (0-∞) [or if not available AUC(0-t) or AUC (0-τ)] will be analysed for periods 1 and 2 utilising a mixed model with regimen fixed effect and subject random effect. Between regimen differences will be computed and back transformed to provide point estimates and 90% CI for the ratios of AUC for GSK3915393 IV + ITZ: GSK3915393 IV. Distributional assumptions underlying the model used for analysis will be examined by obtaining a normal probability plot of the residuals and a plot of the residuals versus the fitted values.

A similar mixed model analysis utilizing period 3-5 data will be conducted to provide point estimates and 90% CI for ratio of AUC for GSK3915393 PO + ITZ: GSK3915393 PO + water. These analyses will also be conducted for log transformed Cmax to derive point estimates and 90% CI for the Cmax ratios of interest.

In addition, descriptive summaries of AUC and Cmax will be produced for each regimen.

4.3.7. Additional Part C Assessments

4.3.7.1. Endpoints/Variables

F, F_A, F_G and F_H, CL, tmax and $t_{1/2}$ following single IV / oral doses of GSK3915393 alone/with water or with ITZ/GFJ

4.3.7.2. Summary Measure

F, F_A, F_G, F_H and CL would be summarized. tmax and $t_{1/2}$ would be summarized by regimen. .

4.3.8. Other Endpoints

4.3.8.1. Endpoints/Variables

Plasma concentration of 4β-hydroxycholesterol and cholesterol, assessed in part B, are considered as a potential *in vivo* marker of CYP3A4 enzyme activity.

4.3.8.2. Summary Measure

The ratio of plasma concentration of 4β-hydroxycholesterol to total cholesterol will be summarised by day and dose (placebo arm will be included). Ratio obtained on day 14 would be divided by the ratio obtained on day 1 to assess the CYP3A4 enzyme activity induction, if 1 indicates no induction.

4.4. Interim Analyses

Details of dose escalation reviews are covered in the dose escalation plan.

4.5. Changes to Protocol Defined Analyses

N/A

5. SAMPLE SIZE DETERMINATION

Twelve, 36 and 12 participants would be randomised to study intervention in parts A, B and C respectively. A further 12 healthy volunteers would be randomised in parts A and B if additional dose levels are required to be studied.

The samples size for part A and B were selected to support the planned assessments of safety and tolerability, the primary objective of parts A and B of the study.

In parts A and B, 9 participants will receive each active oral dose. If 0/9 participants experience a particular adverse event, the upper limit of the exact 95% CI indicates that a true incidence rate of 33.6% could not be ruled out. Whereas if 1/9 participants

experienced an event, the upper limit of the exact 95% CI indicates that a true incidence rate of 48.2% could not be ruled out.

The sample size for part C was selected taking into consideration the precision of estimation of the ratios of AUC and Cmax for the following comparisons of interest.

- GSK3915393 PO + GFJ v GSK3931353 PO + water
- GSK3915393 IV + ITZ v GSK3915393 IV
- GSK3915393 PO + ITZ v GSK3915393 PO + water

Assuming a within subject coefficient of variation of 35%, with 12 subjects the upper and lower limits of the 90% CI for the AUC and Cmax ratios will be 0.78 and 1.28 times the point estimate respectively. Increases in AUC and Cmax are expected to be observed when GSK3915393 is dosed in combination with GFJ or ITZ and it is expected that F_G (ratio of AUC for GSK3915393 PO + water: GSK3915393 PO + GFJ) will be less than 1.

6. SUPPORTING DOCUMENTATION

6.1. Appendix 1 Abbreviations and Trademarks

6.1.1. List of Abbreviations

Abbreviation	Description
AE	Adverse Event
API	Active Pharmaceutical Ingredient
AUC	Area under the concentration time curve
BID	Twice Daily
Cτ	Trough Concentration
CI	Confidence Interval
CL	Clearance
C_{max}	Maximum observed concentration
CPMS	Clinical Pharmacology Modelling and Simulation
CSH	Heterogeneous Compound Symmetry
CS	Compound Symmetry
CV	Co-efficient of variation
CYP3A4	Cytochrome P450 3A4
DEC	Dose Escalation Committee
ECG	Electrocardiogram
F	Absolute bioavailability
FA	Product of fraction of drug absorbed
FG	Fraction of drug escaping gut metabolism
FH	Fraction of drug escaping hepatic metabolism
FTIH	First time in humans
GSK	GlaxoSmithKline
GFJ	Grapefruit Juice
HV	Healthy Volunteer
IV	Intravenous
ITZ	Itraconazole
NQ	Non-quantifiable
OPS	Output and Programming Specification
PBO	Placebo
PK	Pharmacokinetics
QTc	Electrocardiogram QT interval corrected from Heart Rate
RD	Repeat Dose
Ro	Observed Accumulation ratio
Rs	Steady state accumulation ratio

Abbreviation	Description
SAP	Statistical Analysis Plan
SAE	Serious Adverse Event
SD	Single Dose
STD	Standard Deviation
$t_{1/2}$	Apparent terminal half-life
T_{max}	Time to maximum observed concentration
VC	Variance Components
V_d	Volume of distribution
β	Beta

6.1.2. Trademarks

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7. REFERENCES

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GlaxoSmithKline Document number 2020N445405_00 Dose Justification for GK3915393 First Time in Human Study (213585) based on predicted human pharmacokinetic profile and TG2 inhibition

GlaxoSmithKline Document number TMF-11859187 Protocol A Randomized, Placebo Controlled, Double Blind, Single and Repeat Dose Escalation Phase 1 Study to Evaluate Safety, Tolerability, and Pharmacokinetics of GSK3915393 in Healthy Participants and open label assessment of coadministration of GSK3915393 with grapefruit juice and itraconazole on the pharmacokinetics of GSK3915393.

TMF-11886262: 213585 File Note - Changes to feeding regimen and add additional pre dose PK samples in Part B Cohort 2

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