Statistical Analysis Plan

A PHASE 1 STUDY TO ASSESS THE PHARMACOKINETICS AND SAFETY OF ASCENDING DOSES OF JOTROL ORAL GELCAPS IN HEALTHY SUBJECTS, AND TO DETERMINE THE INFLUENCE OF FOOD

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SIGNATURES

Syneos Health Project No.: 202016

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Study Title: A Phase 1 Study, to Assess the Pharmacokinetics and Safety of Ascending Doses of JOTROL Oral Gelcaps in Healthy Subjects, and to Determine the Influence of Food.

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LIST OF ABBREVIATIONS

AE	Adverse Event
aPTT	activated partial thromboplastin time
Ae_{0-t}	Cumulative amount excreted into urinary from time zero to time t
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
AUC_{0-t}	Area under the concentration-time curve from time zero to the last measurable concentration.
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity (extrapolated)
BLQ	Below Limit Of Quantitation
BP	Blood Pressure
bpm	Beats per minute
CI	Confidence Interval
CLsys/F	Apparent systemic clearance
CL_r	Renal Clearance
C_{max}	Maximum Plasma Concentration
CRF	Case Report Form
CV	Coefficient of Variation
ECG	Electrocardiogram
FDA	Food and Drug Administration
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL	High-density lipoprotein
HEENT	Head, Eyes, Ears, Nose, and Throat
HIV	Human Immunodeficiency Virus
HR	Heart Rate
INR	International Normalized Ratio
IV	Intravenous
K_{el}	Elimination rate constant
kg	Kilogram
L	Liter
LDL	Low-density lipoprotein
LS	Least squares
Max	Maximum

MedDRA	Medical dictionary for regulatory activities
MDMA	3,4-methylenedioxymethamphetamine
mg	Milligram
Min	Minimum
min	Minute
mmHg	Millimeters of Mercury
ms	Millisecond
OT	Oral temperature
PCP	Phencyclidine
PK	Pharmacokinetic(s)
PR	PR Interval of the ECG
PTT	Partial Thromboplastin Time
PT	Prothrombin Time
R_{max}	Maximum Rate of Urinary Excretion
RR	RR interval of the ECG
Rsq	r-squared
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SD	Standard Deviation
SE	Standard Error
SOC	System Organ Class
SOP	Standard Operation Procedure
TEAE	Treatment-Emergent Adverse Event
$T_{½\ el}$	Elimination Half-Life
T_{max}	Time of Maximum Concentration
T_{Rmax}	Time of Maximal Urinary Excretion
WHO DD	World Health Organization Drug Dictionary

1. Introduction

This statistical analysis plan (SAP) is intended to give a detailed description of the summaries and the analyses that will be generated for the present study by Syneos Health or a designee. Analyses specified in this plan are based on Jupiter Orphan Therapeutics, Study Protocol No. 202016 (Final version) dated August 20, 2020. Safety, tolerability, and pharmacokinetic (PK) analyses will all be described.

This document defines the populations to be analyzed and provides full details of the statistical analyses, data displays, and algorithms to be used for data derivations to aid in the production of the statistical output. Relevant subject characteristics that will be evaluated are described along with the specific statistical methods.

The plan may change due to unforeseen circumstances and any changes made after the plan has been finalized will be documented. If additional analyses are required to supplement the planned analyses described in the SAP, the changes and justification for the changes will be outlined in a SAP amendment and in the clinical study report (CSR). No change will be made without prior approval of the study sponsor. No revision to the SAP is required for changes that do not affect the statistical analysis methods, definitions, or rules defined in this document.

When applicable, all methodology and related processes will be conducted according to Syneos' standard operating procedures (SOPs) as appropriate. Protocol deviations occurring during the study will be listed.

Shells for all statistical tables, figures and listings referred to in this SAP will be displayed in a separate document.

2. Study Objectives

- To characterize the PK profile of JOTROL (resveratrol) following oral administration of SAD ranging from 200 mg up to a dose currently estimated at 1000 mg, in healthy adult subjects.

- To evaluate the effect of food on the PK profile of JOTROL.

3. Study Design

3.1 General Design

This will be a single center, Phase 1, open-label, sequential SAD study, with a food-effect arm. The study will be divided into two parts:

- Study Part 1 consists of 3 periods with SAD of JOTROL under fasting conditions. Periods 2 and 3 will be initiated with updated doses after safety, tolerability, and PK data are evaluated by the Safety Committee and deemed acceptable for single doses for subsequent doses.
- Study Part 2 consists of a single oral JOTROL dose under fed conditions. JOTROL dose for this study part will derive from study Part 1 safety, tolerability, and PK data.

In order to minimize potential variability, elder subjects will not be included in the food effect part (Part 2).

This study is intended for submision under FDA regulations.

3.2 Study Procedures

The overall schedule of procedures and assessments is provided in the protocol.

3.3 Treatment Description

Treatment A (Period 1) – JOTROL (resveratrol) 100 mg oral gelcap (Jupiter Oprhan Therapeutics, USA) given as 2 x 100 mg gelcaps under fasting conditions, total resveratrol dose: 200 mg.

Treatment B (Period 2) – JOTROL (resveratrol) 100 mg oral gelcap (Jupiter Oprhan Therapeutics, USA) given as 5 x 100 mg gelcaps under fasting conditions, total resveratrol dose: 500 mg.

Treatment C (Period 3) – JOTROL (resveratrol) 100 mg oral gelcap (Jupiter Oprhan Therapeutics, USA) given as 10 x 100 mg gelcaps under fasting conditions, total resveratrol dose: 1000 mg.

Treatment D (Period 4) – JOTROL (resveratrol) 100 mg oral gelcap (Jupiter Oprhan Therapeutics, USA) given as 10 x 100 mg gelcaps under fed conditions, total resveratrol dose: 1000 mg.

3.4 Confinement and Washout

For each period, subjects will be confined from Day -1 until after the 32-hour post-dose blood draw.

There will be a washout of 14 days or more between doses. The washout period may be increased for logistical considerations. Participation of each subject in this study should last approximately 1 month (for subjects participating in study Part 1 only) and 1.5 months (for subjects participating in both study parts).

3.5 Sample Size

A total of 24 healthy adult male or female volunteers will be included in study Part 1. Only 16 subjects who completed study Part 1 will be included in study Part 2. These 16 subjects will be selected according to their order of enrolment in the study, provided their consent to continue the study, they follow the study restrictions and they still meet study criteria. Subjects numbers are judged adequate to achieve the study objectives.

An effort will be made to include to the extent possible, subjects of the following age groups:

• ≥ 65 and ≤ 70 years of age;

• ≥ 70 and ≤ 75 years of age.

3.6 Randomization and Blinding

Subjects will be administered 200 mg, 500 mg and 1000 mg gelcaps sequentially in ascending order. As this is an open-label study no blinding will be applied.

3.7 Subject Withdrawal and Replacement

Subjects will be advised that they are free to withdraw from the study at any time. Over the course of the study, the Sponsor and the Investigator or a designee may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with the clinical site's SOP:

- safety reason;
- non-compliance with protocol requirements;
- significant protocol deviation;
- positive cotinine, alcohol, drug, or pregnancy test;
- vomiting within 3 hours (study Part 1) and 7 hours (study Part 2) after dosing.

Subjects excluded from dosing in one period as per criteria listed above, may be invited to participate in subsequent periods of the study if deemed appropriate by the Investigator and appropriate from a statistical standpoint (i.e. would permit adequate statistical comparison). However, subjects with positive cotinine, alcohol, or drug test will be definitively withdrawn from the study. Hematology results will be reviewed by the Investigator or designee prior to dosing in study Part 2 (Period 4). Subjects will be withdrawn from the study if it is deemed that

the subject's safety may be at risk on the basis of these test results. Subjects who withdraw or are withdrawn from study Part 1 after dosing, for reasons other than safety and tolerability, may be replaced after consultation between the Safety Committee members in order to assure initiating study Part 2 with 16 subjects. Such replacement resulting in dosing more subjects than planned in this protocol would be documented in a protocol amendment.

Subjects who withdraw or are withdrawn will be asked to remain at the clinic until the Investigator or a designee agrees that the subject is fine and can be discharged. As soon as subject withdrawal is confirmed, blood sampling will be stopped. A PK blood draw may be collected at the time of withdrawal if deemed required by the Investigator. Study exit procedures will be performed at the time of withdrawal from the study or as soon as possible thereafter.

4. Change From the Protocol

No changes in planned analyses were done compared to the protocol.

5. Primary and Secondary Parameters

$AUC_{0\text{-inf}}$, $AUC_{0\text{-t}}$ and C_{max} derived from resveratrol, resveratrol-3-glucuronide, resveratrol-4'-glucuronide, and resveratrol-3-sulfate plasma concentrations will be the primary PK parameters. Other PK parameters derived from plasma concentrations (Residual area, T_{max}, $T_{½\ el}$, CL_{sys}/F (resveratrol only) and K_{el}) will be secondary PK parameters (refer to Section 10.3).

For urine PK analyses, $Ae_{0\text{-t}}$, R_{max} and T_{Rmax} parameters will be calculated using urine concentrations (refer to Section 10.4).

Treatment-emergent AEs (TEAEs) will be tabulated by treatment phase. Safety and tolerability data will be reported using descriptive statistics. (refer to Section 9.2).

6. Analysis Populations

The analysis of safety and tolerability parameters will be based on the study population detailed in Section 6.1. The analysis of PK concentration will be based on the study population detailed in Section 6.2. The analysis of PK parameters will be based on the study population detailed in Section 6.3.

6.1 Safety Population

The safety population is defined as all subjects who received at least one dose of the study medication.

6.2 Pharmacokinetic Concentration Population

The PK Concentrations (PC) Population will comprise all subjects who received at least one dose of either study drug and have at least one quantifiable PK concentration.

In addition, if a subject has an episode of emesis within 2 hours of study drug administration, their inclusion in the PK Concentration Population will be determined at the discretion of the pharmacokineticist. The PK Concentration Population will be used for the summaries of all PK concentration data.

6.3 Pharmacokinetic Parameter Population

The PK population will include all subjects completing at least one period and for whom the PK profile can be adequately characterized.

Any subject with pre-dose concentrations will be excluded from the PK and statistical analysis for the respective analyte for the concerned period if the pre-dose concentration is greater than 5% of the C_{max} value of that period for that subject.

Data from subjects who experienced emesis during the sampling interval and who were not withdrawn as per criterion established under section 3.7 may be evaluated after completion of the PK analysis. Any subject who experienced emesis within 3h post-dose will be excluded from the statistical analysis. Data (concentrations and PK parameters) from subjects excluded due to a pre-dose concentration greater than 5% of their C_{max} or from subjects withdrawn due to AEs or vomiting episodes will be presented but excluded from descriptive statistics for the concerned period.

7. Interim Analyses

Interim PK analyses will be performed between each dose level and will inform selection of the subsequent dose. The Interim Analyses using PK concentration data will be done after the bioanalytical phase is completed and will include data from subjects who have finalized their study visits at the time of the interim database availability.

PK parameters will be calculated using scheduled sampling times as actual sampling times will not be available for these interim analyses. Only descriptive statistics will be calculated and provided. Interim descriptive analyses of the PK variables will be conducted for each period (Number of observations [N], arithmetic mean, standard deviation [SD], coefficient of variation [%CV], median, minimum [Min], maximum [Max], geometric mean, and %CV geom.mean).

8. Study Population and Exposure

No inferential analysis will be done. Only observed data will be used.

8.1 Subject Disposition

Subject disposition will be summarized by treatment and overall (frequency and the percentage of subjects). Subject completion and discontinuation information will be listed. In addition, subjects who were dismissed from a period or who did not complete a dosing period will also be presented in this listing, including absence/early discontinuation reason, date and time of discontinuation.

8.2 Protocol Deviations

The protocol deviations will be categorized and listed by subject.

8.3 Demographics and Baseline Characteristics

The descriptive statistics (mean, median, standard deviation [SD], minimum [Min], maximum [Max], and sample size) will be calculated for continuous variables (age, body weight, height and body mass index [BMI]) considering last results (scheduled or unscheduled) obtained at screening. Frequency counts and percentages will be tabulated for categorical variables (age group, sex, ethnicity, and race). Results will be presented summarized by treatment and overall for the safety population and for PK population. All demographic characteristics will be listed by subject.

8.4 Medical History

Medical history at screening will be listed by subject. The Medical Dictionary for Regulatory Activities (MedDRA®), Version 23.1 will be used to classify all medical history findings by System Organ Class (SOC) and Preferred Term (PT).

8.5 Prior and Concomitant Medications

The use of prior and/or concomitant medication will be monitored throughout the study and listed by subject. The World Health Organization Drug Dictionary (WHO DD), Version Sep2020, format B3 will be used to classify all medication reported as from screening through study exit/early termination.

8.6 Study Drug Administration

The study drug administration details (including period, total dose administered, start and end date/time of administration) will be listed by subject for the safety population.

9. Safety Analyses

Safety data will be evaluated for the safety population through the assessment of AEs, laboratory parameters (hematology, biochemistry, serology, coagulation, urinalysis,), 12-lead electrocardiogram (ECG), physical examination, and vital signs assessments. AEs, laboratory values, and vital signs and ECG will be summarized overall as appropriate. Safety data will be summarized, but will not be subjected to inferential analysis.

Details of the additional laboratory work (RNA-Seq Analysis, Iduronidase, mRNA Frataxin, and Other Possible Tests) will be available in a separate protocol.

Scheduled safety measurements will be repeated according to the clinical site SOPs or upon request from the Investigator or designee. Any abnormal repeated measurement will be evaluated by the Investigator or designee and repeated if judged necessary. Further action may be taken upon the Investigator or designee's request.

9.1 Physical Examination Findings

A complete physical examination will be performed at screening. Any abnormal finding prior dosing will be presented in Medical History (MH) and a finding happening after 1st dosing will be categorized as an Adverse Event(AE).

9.2 Adverse Events

AEs will be recorded and evaluated for their seriousness, severity, and relationship to the study medication. AEs will be collected and documented during the course of the study and until 4 days following the last study drug administration, if reported. AEs will be followed-up until complete resolution, or until the Investigator judges it to be safe to discontinue follow-up. The relationship to the study medication will be classified according to the clinical site SOPs.

Treatment-emergent AEs and non-TEAEs (those occurring prior to administration of study medication or that first occurred prior to study drug administration and did not worsen in frequency or severity) will be listed. TEAEs will be defined as AEs that occur on or after the date and time of study drug administration, or those that first occur pre-dose but worsen by increase in occurrence or severity after study drug administration. AEs will also be documented if reported.

The incidence of TEAEs will be summarized using the safety population. The MedDRA® dictionary Version 23.1 will be used to classify all AEs reported during the study by SOC and PT.

Incidence of subjects who experienced TEAEs will be presented by treatment and overall, SOC, PT, by Investigator-assessed relationship and also by severity. Each subject may only contribute once to each of the incidence rates, for a TEAE following a given treatment, regardless of the number of occurrences; the highest severity or highest relationship will be presented, as appropriate. In each table, SOC will be presented in descending order of overall incidence rate in terms of frequency of subjects and then in frequency of events (alphabetical order will be used in case of equal rates). For each SOC, PT will be presented the same way.

Incidence of TEAEs (number of events) will also be presented by period(dose) and overall, by SOC, and PT, by Investigator-assessed relationship and severity.

Frequency of subjects experiencing Serious Treatment Emergent Adverse Events and Suspected Unexpected Serious Adverse Drug Reactions; and the number of events would be also summarized per treatment and overall. Also, these events and Serious Adverse Events (SAEs) will be listed per subject.

The assessment of the relationship of an adverse event with the administration of study drug will be classified according to the clinical site SOPs. The relationship of TEAEs will be classified as probably related, possibly related, remotely related and unrelated (not related) to JOTROL.

9.3 Laboratory Parameters

Biochemistry will be performed at screening and study exit. The following will be assessed: albumin, alkaline phosphatase (ALP), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), urea, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, total bilirubin, and total protein.

Serology will be performed at screening. The following will be assessed: HIV antigen and antibody, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibody.

Hematology will be performed at screening, at check-in of study Part 2 (Period 4), and at study exit. The following will be assessed: complete blood count with differential, hemoglobin, and hematocrit.

Coagulation tests will be performed at screening. The following will be assessed: prothrombin time/international normalized ratio (PT/INR) and activated partial thromboplastin time (aPTT).

Urinalysis will be performed at screening and at study exit. The following will be assessed: macroscopic examination, pH, specific gravity, protein, glucose, ketones, bilirubin, occult blood, nitrite, urobilinogen, and leukocytes. Unless otherwise specified, microscopic examination will be performed according to internal procedures.

Hematology, biochemistry, urinalysis, urine pregnancy test, vital signs (blood pressure [BP], respiratory rate [RR], heart rate [HR], and oral temperature [OT]), ECG, and AE monitoring will be performed on the last study day. For subjects who are not included in study Part 2, study exit procedures will be completed after the last blood draw of Period 3 (after 32 hours post-dose on Day 2). For subjects who are included in both study parts, study exit procedures will be completed after the last blood draw of Period 4 (after 32 hours post-dose on Day 2). If not possible, all efforts will be made to complete study exit procedures within 14 days after the last participation of the subject in the study.

A urine pregnancy test will be performed at screening and at study exit, and a serum pregnancy test will be performed at check-in of each period.

A urine drug screen (amphetamines, methamphetamines, barbiturates, benzodiazepines, tetrahydrocannabinol, cocaine, opiates, PCP, MDMA, methadone) and a urine cotinine test will

be performed at screening. A urine drug screen, a urine cotinine test, and an alcohol breath test will be performed before dosing at check-in of each period.

Listings of all clinical laboratory results will be provided with the abnormal values flagged with "L" (below normal) and "H" (above normal) for continuous parameters, and “A” (abnormal) for categorical parameters, including the evaluation of the abnormal result as clinically significant or not clinically significant.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) for each clinical laboratory test (continuous variables) will be presented for overall. Change from screening for hematology, biochemistry, and urinalysis will be presented. The unscheduled results will not be included in the summary tables. For categorical variable (urinalysis test), the number of subjects (frequency and percentage) will be tabulated by results (e.g., negative, positive, trace …). A summary table of shifts from screening to study exit will be provided. Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

For Serology and Coagulation only listing will be provided.

9.4 Vital Signs

BP, HR, RR and OT will be measured in a sitting position (except for safety reasons) at screening and study exit.
Descriptive statistics (mean, median, SD, Min, Max, and sample size) will be presented by overall for screening and study exit for each vital sign measurement. Descriptive statistics for change from screening to study exit will be presented. Unscheduled results will not be included in the summary tables. Results from post-dose repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A listing of all vital signs results will be provided.

9.5 Safety ECG

Supine 12-lead ECG will be performed at screening and study exit.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) will be presented for screening and study exit for ECG. Unscheduled results will not be included in the summary tables. Change from screening descriptive statistics for study exit will also be presented. Results from repeat tests will not be included in the summary statistics unless the repeat was required (and documented as such) due to technical reasons or an invalid initial result.

A listing of all ECG results will be provided with the abnormal values flagged.

10. Pharmacokinetic Analyses

10.1 Handling of the Below the Lower Limit of Quantification (BLQ) and the No Reportable Concentration

All concentration values that are BLQ occurring prior to dosing as well as samples with no reportable value (NRV) occurring prior to dosing will be replaced by "0.00"; otherwise they (BLQ and NRV) will be set to missing for tabulation, graphical representation and calculation purposes.

10.2 Handling of the Difference between the Scheduled and the Actual Sampling Times

The actual clock time for dosing and the actual clock time for each collection time for the PK samples will be recorded using electronic data capture. For all sampling times, the actual sampling times will be calculated as the difference between the sample collection actual clock time and the actual clock time of dosing. The actual post-dose sampling times expressed in hours and rounded off to three decimal digits will be used to calculate the PK parameters, except for pre-dose samples occurring prior to dosing, which will always be reported as zero (0.000), regardless of the time difference. Scheduled sampling times will be presented in concentration tables and mean graphs, while actual sampling times will be presented in the individual graphs in the PK section of the report. A listing of the actual times for PK sampling will be provided for PK samples.

10.3 Pharmacokinetic Parameters

PK analyses will be performed using Phoenix® WinNonlin® version 8.2, which is validated by Syneos Health. Inferential statistical analyses will be performed using Statistical Analysis System (SAS®) version 9.4 according to FDA guidelines. Bioanalysis of all samples should be completed prior to the initiation of the PK statistical analyses.

A total of 17 blood samples will be collected in each period for PK analyses:
pre-dose and 0.133, 0.250, 0.500, 1.00, 1.50, 2.00, 3.00, 4.00, 5.00, 6.00, 8.00, 10.0, 12.0, 16.0, 24.0, and 32.0 hours post- dose.

Plasma PK Parameters

The following PK parameters will be calculated by standard non-compartmental methods with JOTROL (resveratrol) plasma concentrations:

Primary parameter:

$AUC_{0\text{-inf}}$: Area under the concentration-time curve from time zero to infinity (extrapolated), calculated as $AUC_{0\text{-t}} + C_t/K_{el}$, where C_t is the last observed measurable concentration.

AUC_{0-t}:	Area under the concentration-time curve from time zero to the last measurable concentration. AUC_{0-t} will be calculated using the linear trapezoidal-linear interpolation method.
C_{max}:	Maximum observed concentration.

Secondary parameters:

Residual area:	Residual area, calculated as 100*(1- AUC_{0-t} / AUC_{0-inf}).
T_{max}:	Time of observed C_{max}.
$T_{½\ el}$:	Elimination half-life, calculated as ln (2)/K_{el}.
K_{el}:	Elimination rate constant. This parameter will be the negative of the estimated slope of the linear regression of the ln-transformed concentration versus time profile in the terminal elimination phase. Best fit method will be used to calculate the K_{el} from at least 3 concentration data points excluding the C_{max}. Rsq adjusted, the goodness of fit statistic for the terminal elimination phase, adjusted for the number of points used in the estimation of K_{el} must be ≥ 0.8. No points below Tmax will be used. If the K_{el} cannot be measured (e.g.: fewer than 3 non-zero concentrations in the terminal elimination phase or Rsq adjusted < 0.8), the PK parameters derived from K_{el} will not be reported for that individual PK profile (AUC_{0-inf}, Residual area, and $T_{½\ el}$). The timepoint where ln-linear K_{el} calculation begins ($K_{el\ Lower}$) and the actual sampling time of the last measurable concentration used to estimate the K_{el} ($K_{el\ Upper}$), as well as the Rsq adjusted for the ln-linear regression for the calculation of the elimination rate constant will be reported.

Additional PK analyses may be performed. Upon Sponsor's request, PK repeats might be performed according to Syneos Health's SOP. If re-assays are requested for PK reasons (not advised) , final results will include re-assay values, while results with original values will be presented in an appendix of the clinical study report as supportive data. The rationale for re-assays for PK reasons must be provided.

10.4 PK Parameters Calculated With Urine Concentrations

In study Part 1, urine will be collected for quantitation for PK analysis. Urine samples will be collected at 6 times or time intervals: spot pre-dose (within 2 hours before dosing), 0-4 hours, 4-8 hours, 8-12 hours, 12-24 hours, and 24-32 hours post-dose.

Urine samples will be used to calculate the following parameters for resveratrol, resveratrol-3-glucuronide, resveratrol-4'-glucuronide, and resveratrol-3-sulfate:

Ae_{0-t}:	Cumulative urinary excretion from time zero to time t, calculated as the sum of the amounts excreted over each collection interval. The amount excreted in urine for each time interval is calculated as the urine concentration multiplied by the urine volume.

R_{max}:	Maximum rate of urinary excretion, calculated by dividing the amount of drug excreted in each collection interval by the time over which it was collected.
T_{max}:	Time of R_{max}, calculated as the midpoint of the collection interval during which R_{max} occurred.
CL_r:	Renal clearance, calculated as $Ae_{0\text{-}t}/AUC_{0\text{-}t}$ (plasma) where t is T_{last}

10.5 Statistical Analyses

10.5.1 PK Parameters in Plasma

Individual and mean plasma concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (number of observations, arithmetic and geometric means, SD, coefficients of variation [CV%], Min, Max, and median) of the plasma concentrations versus time data will be presented by Treatment as well for the PK parameters. Summary statistics will be used to describe the PK profile for each treatment.

10.5.2 Assessment of Dose Proportionality

Dose proportionality analysis for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} will be performed (using the power model with mixed procedure from SAS®) considering data under fasting conditions (Periods 1, 2, and 3).

Power model will include the PK parameter as the response variable and dose (mg) as the explanatory variable. For this model, the variable dose will be treated as a continuous variable.

The form of the model is as follows:

PK Parameter = $a \times \text{Dose}^b \times e^{\epsilon}$, where Dose ≥ 0, and e^{ϵ} represents the associated error.

Thus, perfect dose proportionality is met when b=1 (ignoring error). This becomes a linear relationship following a natural-log transformation, The mixed effect statistical model will be the following:

$$\ln(P_{ij}) = \ln(a) + b \cdot \ln(Dose_{ij}) + s_i + \varepsilon_{ij},$$

where P_{ij} is the value in the j^{th} period for the i^{th} subject for the PK parameter analyzed, $Dose_{ij}$ is the dose in the j^{th} period for the i^{th} subject, s_i is the random effect associated to the i^{th} subject, and ε_{ij} is the random error in the j^{th} period for the i^{th} subject. The s_i and ε_{ij} values are assumed to be mutually independent and normally distributed.

The estimate of b together with a 90% confidence interval (CI) will be provided (for each PK parameter model), and this will be used to quantify dose proportionality.

```
proc mixed data=dataset;
class subject;
model ln_pk_parameter = ln_dose / solution;
random subject;
estimate 'Beta Estimate' ln_dose 1 / cl alpha=0.1;
ods output estimates = estimates;
run;
```

The PK parameter values estimated from the power model will be plotted against dose. This plot will also include individual subject values ± SD (separately by dose level).

10.5.3 Criteria for Determination of Dose-Proportionality

PK dose proportionality will be concluded if the 90% C.I. of *b* for $AUC_{0\text{-}inf}$, $AUC_{0\text{-}t}$ and C_{max} are entirely contained within the following bounds that depend on the dose range ratio (Smith BP, 2000) (i.e., ln (1000/200) = ln (5)):

- Lower bound = 1+ln (0.80)/ln (5) = 0. 861353116 and
- Upper bound = 1+ln (1.25)/ln (5) = 1.138646884

10.5.4 Food-Effect assessment

For evaluation of the food-effect, PK data (ln-transformed $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, C_{max} and untransformed T_{max}) reported under fed conditions (Period 4) and under fasting conditions (for the same dose level) will be compared using ANOVA from SAS®. The ratio (fed/fasting) and 90% geometric confidence interval will also be calculated for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max}.

If 90% CI for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} are within 80.00% to 125.00% then no food effect will be concluded.

For all analytes, using GLM procedure in SAS, ANOVA will be performed on ln-transformed $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$ and C_{max} at the alpha level of 0.05. The model will include Treatment as fixed effect and Subject as a random effect. Inter- and intra-subject coefficient of variation will be estimated. The ratio of geometric means and 90% confidence interval for the ratio of geometric means, based on least-squares means from the ANOVA of the ln-transformed data, will be calculated for $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, and C_{max}.

SAS® codes for ANOVA: fed vs. fasting

```
PROC GLM data=basepk;
CLASS TREATMENT SUBJECT;
MODEL VAR = TREATMENT SUBJECT;
RANDOM SUBJECT;
LSMEANS TREATMENT / cl pdiff alpha=0.1;
ESTIMATE 'fed - fasting' TRT 1 -1/cl alpha=0.1;
run;
```

Additional statistical analysis may be performed.

10.5.5 Urine PK analysis

Summary statistics will be used to describe urinary excretion of resveratrol, resveratrol-3-glucuronide, resveratrol-4'-glucuronide, and resveratrol-3-sulfate.
Additional statistical analysis may be performed.

11. Percentages and Decimal Places

If not otherwise specified, the following rules will be applied, with the exception of PK tables and listings described below:

Percentages will be presented to one decimal point.

Percentages equal to 0 or 100 will be presented as such without a decimal point.

Minimum and maximum will be presented with the same precision as the original values and, mean, standard deviation, and median will be presented with one more decimal place than the original values.

All digits will be used for pharmacokinetic and statistical PK calculations. For PK tables and listings, the final reportable results or data will be presented by rounding off to two decimal digits, except for the following situations (this applies to individual data and descriptive statistics):

K_{el} and Rsq adjusted data: rounded off to four decimal digits.

PK parameter related to time such as T_{max}, must be reported with the same precision as the actual sampling time: rounded off to 3 decimal digits.

Concentration versus time data, as well as C_{max}: reported as they appear in corresponding dataset.

12. Handling of Missing Data

For safety,

- If an AE is recorded with an onset date corresponding to a dosing day, but the time is missing, then the AE will be assigned to the treatment phase planned treatment with a dosing planned that day.

- If an AE is recorded with an onset date that does not correspond to a dosing day, but the time is missing, then the AE will be assigned to the treatment phase planned treatment that covers the AE onset day.

- If an AE is recorded with an onset date where day and time are both missing, then the AE allocation to a treatment phase planned treatment will be done on a case by case basis considering available information (e.g. AE onset date, AE end date, AE comments, subject disposition).

For PK, only observed data will be used in the data analysis except for concentration values BLQ as described in Section 10.1. No attempt will be made to extrapolate or interpolate estimates for missing data.

13. Data Handling

The PK plasma concentrations, safety, and tolerability data will be received as SAS® datasets from the Syneos Health data management facility. Screening failures and ineligible volunteer's data (subject disposition) will be received from the clinical site as source data.

14. Software to be Used

PK analysis will be performed using Phoenix WinNonlin® version 8.2 or higher, which is validated for bioequivalence/bioavailability studies by inVentiv. The safety data tables and listings, as well as PK tables and listings will be created using SAS®, release 9.2 or a higher version. PK figures will be created using R version 3.5 (or higher). The CSR will be created using Microsoft® Office Word 2010, or a higher version.

15. Reference List

- Smith BP, Vandenhende FR, DeSante KA, et al. Confidence interval criteria for assessment of dose proportionality. Pharmaceutical research 2000;17:1278-83.