Official Title:	**An Open-Label Study to Assess the Safety, Pharmacokinetics, and Pharmacodynamics of Treprostinil Palmitil Inhalation Powder in Participants with Pulmonary Arterial Hypertension**
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STATISTICAL ANALYSIS PLAN

An Open-Label Study to Assess the Safety, Pharmacokinetics, and Pharmacodynamics of Treprostinil Palmitil Inhalation Powder in Participants with Pulmonary Arterial Hypertension

Study Number: INS1009-201

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CONFIDENTIALITY STATEMENT

Approval

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This Statistical Analysis Plan has been reviewed and approved by the Insmed Incorporated (hereinafter referred to as Insmed) representatives listed below.

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1. LIST OF ABBREVIATIONS

Table 1: List of Abbreviations

Abbreviation	Term
6MWD	6-minute walk distance
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
AUC	area under the plasma concentration versus time curve
AUC_{0-inf}	area under the plasma concentration versus time curve from time zero to infinity
AUC_{0-t}	area under the plasma concentration versus time curve from time zero to time of last quantifiable concentration
BLQ	below the limit of quantification
BMI	body mass index
BSA	body surface area
C_{max}	maximum (peak) plasma concentration of study drug
CI	cardiac index
CL/F	apparent total clearance of study drug from plasma after extravascular administration
CO	cardiac output
CO_2	carbon dioxide
CPK	creatine phosphokinase
CSR	clinical study report
CT	computerized tomography
eCRF	electronic case report form
ECG	electrocardiogram
EOT	end of treatment
$ETCO_2$	end tidal carbon dioxide concentration
ETO_2	end tidal oxygen concentration
EUT	extended use treatment
GCP	Good Clinical Practice
hCG	human chorionic gonadotropin
HBsAg	hepatitis B surface antigen
HIV	human immunodeficiency virus
ICF	informed consent form

Abbreviation	Term
ICH	International Council for Harmonisation
IMP	investigational medical product
INR	international normalized ratio
LVEF	left ventricular ejection fraction
LDH	lactate dehydrogenase
MedDRA	Medical Dictionary for Regulatory Activities
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MVO_2	mixed venous oxygen saturation
n	number of participants
PAH	pulmonary arterial hypertension
PAP	pulmonary arterial pressure
PCWP	pulmonary capillary wedge pressure
PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PT	preferred term
PTT	partial thromboplastin time
PVR	pulmonary vascular resistance
QD	once daily
RAP	right atrial pressure
RHC	right heart catheter
RVP	right ventricular pressure
SAP	statistical analysis plan
SD	standard deviation
SOC	system organ class
SPO_2	arterial blood oxygen saturation
SRC	safety review committee
$t_{1/2}$	elimination half life
TAPSE	tricuspid annular plane systolic excursion
TEAE	treatment-emergent adverse event
t_{max}	time to maximum (peak) plasma concentration following study drug administration

Abbreviation	Term
TP	treprostinil palmitil
TPIP	dry powder for inhalation formulation of treprostinil palmitil
TRE	Treprostinil
TVR	tricuspid valve regurgitation
ULN	upper limit of normal
Vd/F	apparent volume of distribution at terminal phase
VCO_2	carbon dioxide production
VE/VCO_2	minute ventilation-CO_2 production ratio
WHO	World Health Organization

2. INTRODUCTION

The purpose of this statistical analysis plan (SAP) is to define the planned statistical analysis of the study data to be included in clinical study report (CSR). This SAP is written based on study protocol INS1009-201 amendment 2, Version 3.0, dated 03 Mar 2022.

The reader is encouraged to read the study protocol for details on the conduct of this study, the operational aspects of clinical assessments, and the timing for a participant to complete the participation in this study.

The SAP is intended to be in agreement with the study protocol. However, the SAP may contain more details or other types of analyses (e.g., other endpoints). When differences exist in descriptions or explanations provided in the study protocol and this SAP, the SAP prevails; the differences will be explained in the CSR.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Primary Objective and Endpoints

Objective	Endpoint
1. To evaluate the safety and tolerability of single doses of dry powder for inhalation formulation of treprostinil palmitil (TPIP) in participants with pulmonary arterial hypertension (PAH)	• Frequency of treatment-emergent adverse events (TEAEs)

3.2. Secondary Objectives and Endpoints

Objective	Endpoint
1. To evaluate the effects of single doses of TPIP on pulmonary vascular resistance (PVR) in participants with PAH over the first 24 hours following administration	• Change from Baseline in PVR at 8 and 24 hours after TPIP administration
2. To evaluate the pharmacokinetic (PK) of treprostinil (TRE) in participants with PAH	• C_{max}, t_{max}, $AUC_{t1–t2}$, $AUC_{0–inf}$, $t_{1/2}$ of TRE in plasma

3.3. Exploratory Objectives and Endpoints

Objective	Endpoint
1. To evaluate the pharmacodynamic (PD) effects of single doses of TPIP in participants with PAH over 24 hours following administration	• Change from Baseline in PVR, PAP, RVP, RAP, CO, oxygen consumption, MVO_2, SpO_2, heart rate, systemic blood pressure, hemoglobin, at selected timepoints after TPIP administration
2. To evaluate the effect of single doses of TPIP on selected biomarkers in participants with PAH over 24 hours following administration	• Change from Baseline in selected biomarker concentrations at selected time points after TPIP administration
3. To evaluate changes in clinical laboratory parameters in participants with PAH after TPIP administration	• Clinically relevant change from Baseline in hematology, coagulation, and clinical chemistry parameters (Section 10.2, Table 5 of study protocol)
4. To evaluate the PK of treprostinil palmitil (TP) in participants with PAH To evaluate the PK of TRE in participants with PAH	• Plasma PK parameters of TP, such as C_{max}, t_{max}, $AUC_{t1–t2}$, $AUC_{0–inf}$, $t_{1/2}$, CL/F and Vd/F • Plasma PK parameters of TRE, such as C_{max}, t_{max}, $AUC_{t1–t2}$, $AUC_{0–inf}$, $t_{1/2}$, CL/F and Vd/F
5. To evaluate the effects of TPIP on resting respiratory gas exchange in participants with PAH[a]	• Change from Baseline in respiratory rate, minute ventilation, VCO_2, ETO_2, $ETCO_2$, SpO_2 over 24 hours after TPIP administration
6. To evaluate the effects of TPIP on parameters of right ventricular function in participants with PAH as measured by transthoracic echocardiogram[b]	• Change from Baseline in LVEF, TVR maximum velocity, TAPSE, and end diastolic right ventricular volume at 2 to 8 hours after TPIP administration
7. To evaluate the effects of TPIP on pulmonary vasculature and blood flow parameters in participants with PAH as measured by pulmonary CT scan[c]	• Change from Baseline in total blood volume, blood volume in vessels < $5mm^2$ at 4 to 8 hours after TPIP administration

[a] Respiratory gas exchange testing optional at Investigator discretion

[b] Echocardiogram optional at investigator discretion
[c] Pulmonary CT scan optional at investigator discretion
AUC_{0-inf} = area under the concentration time curve from time zero to infinity; AUC_{t1-t2} = area under the concentrationtime curve from time zero to last sampling timepoint with measurable concentration; C_{max} = maximum observed concentration after drug administration; CL/F = apparent total clearance; CO = cardiac output; CT = computerized tomography; $ETCO_2$ = end tidal carbon dioxide concentration; ETO_2 = end tidal oxygen concentration; LVEF = left ventricular ejection fraction; MVO_2 = mixed venous oxygen saturation; PAP = pulmonary arterial pressure; RAP = right atrial pressure; RVP = right ventricular pressure; SpO_2 = arterial blood oxygen saturation; TAPSE = tricuspid annular plane systolic excursion; TVR = tricuspid valve regurgitation; t_{max} = time of maximum observed concentration following drug administration; $t_{1/2}$ = elimination half-life; Vd/F = apparent volume of distribution at terminal phase; VCO_2 = carbon dioxide production

3.4. Exploratory Objectives and Endpoints for the Optional Extended Use Treatment Period

Objective	Endpoint
1. To evaluate the safety and tolerability of TPIP in participants with PAH	• Frequency of TEAEs during extended use treatment (EUT)
2. To assess the effect of TPIP on exercise capacity	• Change from Baseline in 6MWD distance at EUT Baseline to EUT Week 16

6MWD = 6-minute walk distance

4. STUDY DESIGN

4.1. Summary of Study Design

This is a Phase 2a, open label study to assess the safety, tolerability, PD effects and PK of TPIP administered to participants with PAH (approximately 6 to 10 evaluable participants are planned for the study). This is the first study of TPIP in participants with PAH. Each participant will receive a single dose of TPIP. The first participant will receive a dose containing 112.5 µg of TP. The TP dose level for each subsequent participant will be determined following review and adjudication of all available safety, PK, and PD data from the previous participant(s) by the safety review committee (SRC) (Section 1.3.2.1 of study protocol).

This is a "proof of mechanism" study designed to investigate the following:

1. The safety and tolerability of TPIP in participants with PAH;
2. The relationship between the PK and PD effects of TPIP in participants with PAH, and

3. The duration of the PD effects of a single dose of TPIP in participants with PAH.

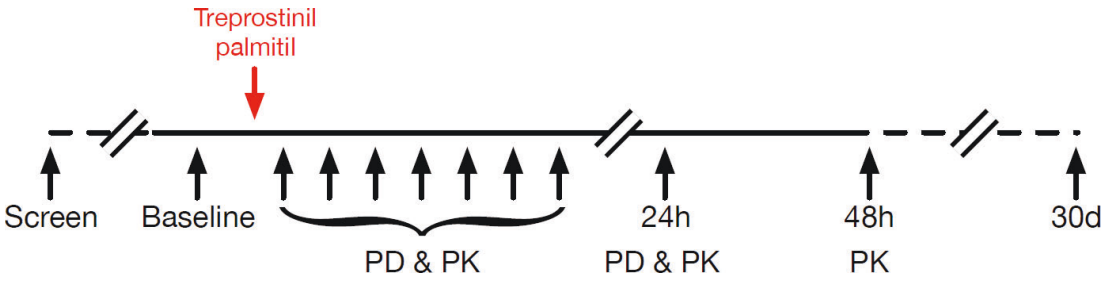
The cardiopulmonary and overall functional status of patients with PAH can be labile and medical instability can develop quickly. The study is designed to provide maximal safety and minimal risk to participants. As such, there is considerable flexibility built into the protocol to allow Investigator discretion with the frequency, timing, and/or conduct of PD and PK assessments to ensure participant safety. Because the study requires an overnight inpatient treatment period, Institutional requirements for elective inpatient admissions, which may vary from site to site, will be followed.

For individual participants, the study will consist of an outpatient screening period lasting up to 30 days. The inpatient treatment period will start on Study Day 1 and is expected to last overnight into Study Day 2. Outpatient PK sampling and safety follow-up at 48 (± 4) hours post TPIP dose will extend into Study Day 3. A remote safety follow-up visit is scheduled for Study Day 30 (± 2 days). The 48 hour PK visit will also include safety assessments including electrocardiogram (ECG), clinical laboratory evaluations, vital signs, physical examination, and adverse event (AE) collection.

An optional extended treatment period of 16 weeks will be available to participants who have completed the single-dose inpatient treatment period. There will be a second safety follow-up visit 30 days (± 3 days) after EUT Week 16 for participants who elect to enter the EUT period. If the initial remote safety follow-up visit overlaps with the start of the extended treatment period, the initial remote safety visit will be shortened and conducted just prior to administration of investigational medical product (IMP) in the extended treatment period.

The procedures and assessments conducted at each study visit in the study are provided in Table 1 to 6 of the study protocol. Please refer to Figure 1 and Figure 2 for a schematic diagram of the study design.

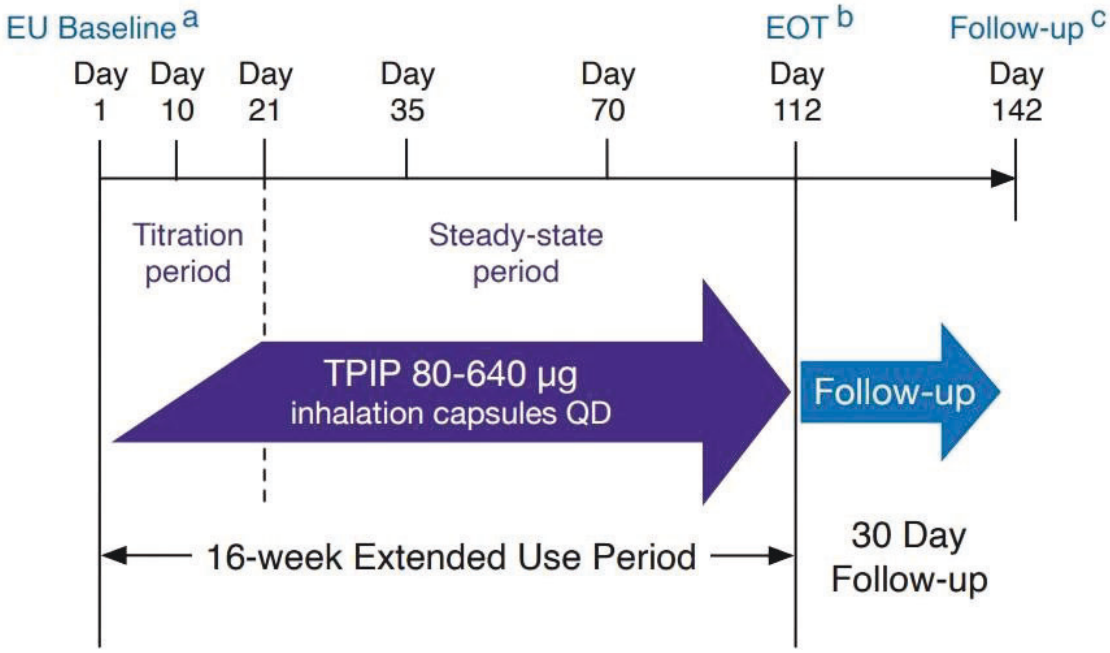
Figure 1: Study Design, Participant Progression Through INS1009-201 Study[a]



h=hours; d=days

[a] Does not include optional EUT period; the 30 day follow-up visit will be shortened and conducted just prior to administration of IMP in the extended treatment period if the visit overlaps with the start of the extended treatment period.

Figure 2: Participant Progression Through the Optional Extended Use Treatment Period



[a] The 30 day follow-up visit from the single dose period will occur on EUT Day 1 if the visit overlaps with the start of the extended treatment period.
[b] At the Investigator's discretion, participants may undergo a study drug taper for up to 2 weeks after the Week 16 visit.

[c] The follow-up telephone call or visit will be 30 days after the Week 16 visit. QD = once daily; EOT = end of treatment

4.2. Study Intervention/Treatment

4.2.1. Inpatient Treatment Period (Single Dose)

A single dose of TPIP will be used in the inpatient treatment period, as listed below. A dose may consist of a single capsule or of multiple capsules administered in succession.

The study drug will be administered by oral inhalation using a Plastiape capsule-based Dry Powder Inhaler (RS 01 Mod 7).

Name of Treatment	Dose	Supplied Formulation	Route and Frequency of Administration
TPIP	One or more single actuation capsules	Single actuation capsules containing 112.5 µg TP per 7.5 mg powder	Inhalation; single dose after Baseline assessments
TPIP	One or two single actuation capsules (80 µg, 160 µg, 240 µg, 320 µg, 400 µg, 480 µg, or 640 µg)	Dry powder single actuation capsules containing 80 µg, 160 µg, or 320 µg	Inhalation; single dose after Baseline assessments

4.2.2. Optional Extended Use Treatment Period

The IMP used in the EUT period will be capsules containing 1 of 3 dosage strengths of TPIP (80 µg, 160 µg, or 320 µg). IMP should be taken around the same time every day. Administration of IMP will be via inhalational dry powder packaged in single actuation capsules. One or 2 capsules will be administered for each dose. Administration of 2 capsules should be done sequentially.

Each TPIP capsule contains 8 mg, 16 mg, or 32 mg of dry powder containing 80 µg, 160 µg, or 320 µg of the TP prodrug, respectively.

The study drug will be administered by oral inhalation using a Plastiape capsule-based Dry Powder Inhaler (RS 01 Mod 7).

Name of Treatment	Dose	Supplied Formulation	Route of Administration
TPIP	One or two single actuation capsules (80 µg, 160 µg, 240 µg, 320 µg, 400 µg, 480 µg or 640 µg QD)	Dry powder single actuation capsules containing 80 µg, 160 µg, or 320 µg	Inhalation (QD)

4.3. Sample Size Determination

The sample size for this study is not based on statistical power calculations. The planned number of evaluable participants to complete the study is approximately 6-10.

4.4. Randomization

There is no randomization in this study.

5. PLANNED ANALYSES

5.1. Interim Analyses

No interim analysis is planned for this study.

5.2. Final Analyses

Final analysis is planned to be conducted after database lock of the study.

The study was terminated by Insmed. At time of study termination only 1 subject was enrolled into the study and treated. Therefore the collected study data will only be listed, there will be no summaries.

The sections below describing the planned statistical analysis, which was planned for the full study population according to study protocol.

5.3. Safety Review Committee Review

All available safety, PK, and PD data from each participant will be evaluated by the SRC prior to the next participant in order to determine the value of proceeding with the study and the TPIP dose for each subsequent participant.

6. ANALYSIS SETS

6.1. Safety Analysis Set

All participants who receive a single dose of TPIP will be considered evaluable for safety and included in the safety population.

A separate safety population for participants of EUT will be determined.

6.2. Pharmacokinetic Analysis Set

All participants who receive a single dose of TPIP and have at least one measurable post-dose plasma TP/TRE concentration will be considered evaluable for PK and included in the PK population.

6.3. Pharmacodynamic Analysis Set

All participants who receive a single dose of TPIP and have a baseline datapoint and at least one measurable post-dose PD datapoint will be considered evaluable for PD and included in the PD population.

6.4. Biomarker Analysis Set

All participants who receive a single dose of TPIP and have at least one measurable post-dose biomarker datapoint will be considered evaluable for biomarkers and included in the biomarker population.

A separate biomarker population for participants of EUT will be determined.

7. GENERAL STATISTICAL CONSIDERATIONS

Due to the limited number of participants in the study and planned dose escalation after each participant, the observed study data will be presented in by-participant listings. By-participant listings will comprise of all observed data, e.g., single-dose inpatient treatment period and EUT. For selected data points, summaries will be provided only for an overall group. If three or more participants have received the same dose of study drug, summary tables will be provided for those dose group(s).

All statistical analyses will be conducted using statistical analysis system SAS® Version 9.4 or higher (SAS Institute, Cary, NC).

Descriptive statistics for continuous variables include n, mean, standard deviation (SD), median, minimum, maximum, 1st quartile and 3rd quartile (if required). Summaries of continuous variables that have some values recorded using approximate values (e.g., < or >) will use the numeric part of the value in calculations. Listings will present the data in its original format.

Categorical variables will be tabulated by counts and by percentage of participants in corresponding categories. Footnotes will specify the basis for the percentages.

Data from unscheduled visits will not be used in summaries, except for the derivation of baseline.

Listings corresponding to all summaries in this section will be provided. Listings will show data from scheduled and unscheduled visits.

7.1. Baseline Definition

Baseline for single-dose inpatient treatment period will be defined as the last non-missing assessment (including repeated and unscheduled assessments) before the first study drug administration, unless otherwise specified.

Baseline for EUT will be defined as the assessment at EUT Day 1 visit.

The start of EUT period is defined as the date of EUT Day 1 visit.

Change from baseline will be calculated as:

- Change from baseline = post baseline value – baseline value.

7.2. Handling of Missing Data

The imputations described in the following sections will be performed after all efforts fail to obtain the data.

All other data will be reported on available values and no imputations will be performed.

7.2.1. Missing Start and Stop Dates for Prior and Concomitant Medication

The rules to categorize medications to 'Prior' or 'Concomitant' are described in Section 8.4.1. Missing or partially missing start or stop dates will be not imputed.

7.2.2. Missing Start and Stop Dates for Adverse Events

The rules to categorize AEs to 'TEAE' or 'non-TEAE' are described in Section 10.2. Missing or partially missing start or stop dates will be not imputed.

7.3. Derived and Transformed Data

7.3.1. Durations

A duration between one date (*date1*) and another later date (*date2*) is calculated using the following formula:

- Duration (days) = *date2* – *date1* + 1
- Duration (weeks) = duration (days) / 7.

7.3.2. Study Day

Study Day is calculated as date of event minus the date of first exposure to study drug plus one (1) if the date of event is on or after the date of first exposure to study drug.

If the date of event is before the date of first exposure to study drug, the Study Day will have negative values and is calculated as (date of event minus the date of first exposure to study drug).

Consequently, there is no Study Day zero (0).

8. STUDY POPULATION SUMMARIES

The safety analysis set will be used for all study population summaries unless otherwise stated.

8.1. Participants Disposition

For participants screened (participants who have signed the informed consent) the following will be summarized for overall (if applicable also by dose group):

- the number of participants who are screened;
- the number of participants who continue into the study (number of participants who are not screen failure);
- the number of participants who failed screening and the reasons for screen failure;
- the number of participants who are treated in single-dose inpatient treatment period;
- the number of participants who completed the study (single-dose inpatient treatment period);
- the number of participants who discontinued treatment and the reason for discontinuation (single-dose inpatient treatment period);
- the number of participants who discontinued from study and the reason for discontinuation (single-dose inpatient treatment period);

- the number of participants in EUT;
- the number of participants who completed the EUT;
- the number of participants who discontinued treatment in EUT and the reason for discontinuation;
- the number of participants who discontinued from EUT and the reason for discontinuation;
- the number of participants in each analysis population.

A separate summary (if applicable) will be provided for subjects screened for EUT.

Participant disposition data will also be presented in a by-participant listing for screened participants.

8.2. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the protocol. An important deviation (referred to as a major deviation) is a subset of protocol deviations that leads to a participant being discontinued from the study, or significantly affects the participant's rights, safety, or well-being or the completeness, accuracy, and reliability of the study data. An important deviation can include nonadherence to inclusion or exclusion criteria or nonadherence to Food and Drug Administration regulations or International Council for Harmonisation (ICH) E6(R2) guidelines.

Protocol deviations will be documented by the clinical monitor throughout the course of monitoring visits. The investigator will be notified in writing by the monitor of deviations. The Institutional Review Board should be notified of all protocol deviations, if appropriate, in a timely manner.

All protocol deviations will be presented in a by-participant listing, including the categorization of the deviation as major or not.

8.3. Demographics, Baseline Characteristics, Medical History

8.3.1. Demographics

The demographic characteristics consist of age (years), sex, race, and ethnicity. Baseline characteristics were observed in terms of screening weight (kg), height (cm), body mass index (BMI) (kg/m2), body surface area (BSA) (m^2), and tobacco use.

Participant demographic and baseline characteristics will be summarized for overall treatment group (if applicable also by dose group) and presented in a by-participant listing.

A separate summary (if applicable) and listing will be provided for safety analysis set of EUT.

8.3.2. Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA, Version 25.0).

Medical history will be summarized by system organ class (SOC) and preferred term (PT) for overall treatment group (if applicable also by dose group) and presented in a by-participant listing.

8.4. Medications and Treatments

8.4.1. Prior, Concomitant and Post Medications

Information regarding prior medications taken by the participant within the 30 days before signing the informed consent form (ICF) will be recorded in the participants electronic case report form (eCRF).

Any concomitant medication deemed necessary for the welfare of the participant during the study may be given at the discretion of the investigator. If a concomitant medication is taken, except for those specified in the protocol, a joint decision will be made by the investigator and the sponsor to continue or discontinue the participant based on the time the medication was administered, its pharmacology and PK, and whether the use of the medication will compromise the safety of the participant or the interpretation of the data. The investigator is responsible for ensuring that details regarding the medication are adequately recorded in the eCRF.

Prior, concomitant and post medications will be coded using the latest version of the World Health Organization (WHO) Drug Dictionary (202203).

Concomitant medications are those medications taken on or after the first dose of study drug, i.e., the end date is on or after the date of first study drug dosing (but before 30 days after last dose of study drug) or ongoing. If the end date of a medication is partially missing, and the possible interval for the end date includes the date of first study drug dosing or the date of 30 days after last dose of study drug dosing, the medication is considered 'Concomitant'.

Post medications are those medications taken on or after the date of 30 days after last dose of study drug, i.e., the start date is on or after 30 days of last study drug dosing.

Prior medications are those medications taken before the first dose of study drug. A medication that starts prior to first dose of study drug but continues after the first dose of study drug is classified both in prior and concomitant medications. If the medication continues after the date of 30 days after last study drug dosing, the medication will be also classified as ‘Post’. A medication is classified as 'Prior' if the eCRF question 'Started prior to study drug' is answered ‘Yes’.

All prior, concomitant and post medications will be summarized for overall treatment group (if applicable also by dose group) and presented in a by-participant listing.

8.4.2. Medical and Surgical Treatment Procedures

All medical and surgical treatment procedures will be coded using MedDRA, Version 25.0. All medical and surgical treatment procedures will be presented in a by-participant listing.

8.5. COVID-19 Impact

Potential COVID-19 impact is captured in the eCRF and will be summarized and listed as part of respective summaries or by-participant listings.

Disposition summary and listing will include:

- Screen failure related to COVID-19;
- Early termination of study related to COVID-19.

AE overview table and listings will include:

- AEs that fall into the category of COVID-19 related.

Listing of protocol deviations will include:

- Protocol deviations due to COVID-19.

9. SAFETY AND EFFICACY

9.1. Primary Endpoint

The analysis of primary endpoint in terms of frequency of TEAEs will be described in Sections 10.2 to 10.3 of this SAP.

9.2. Secondary Endpoints

9.2.1. Evaluation of Change in Pulmonary Vascular Resistance

PVR and the change from baseline in PVR at 8 and 24 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) for the PD population. All measurements of PVR will be included in a by-participant listing.

9.2.2. Evaluation of Pharmacokinetics of Treprostinil

The analysis of TRE plasma PK parameter of C_{max}, t_{max}, $AUC_{t1–t2}$, $AUC_{0–inf,}$ $t_{1/2}$ will be described in Section 11.

9.3. Exploratory Objectives

9.3.1. Evaluation of Pharmacodynamics

PD assessments of PAP, RVP, RAP, CO, oxygen consumption, MVO_2, SpO_2, (from right heart catheter (RHC)), heart rate, systemic blood pressure, hemoglobin, pulmonary capillary wedge pressure (PCWP), cardiac index (CI) and changes from baseline at 8 and 24 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) for the PD population. In a by-participant listing all observed time points together with their changes at 8 and 24 hours after TPIP administration will be listed.

Individual profiles using planned sampling times will be provided for PD assessments (all participant profiles of one parameter will be shown in one plot, [spaghetti plot]).

9.3.2. Evaluation of Selected Biomarker

Biomarker N-terminal (NT)-pro hormone brain natriuretic peptide (NT-pro-BNP) and changes from baseline at 4 and 8 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) for the biomarker population. In a by-participant listing all observed time points together with their changes at 4 and 8 hours after TPIP administration will be listed.

A separate summary (if applicable) and listing will be provided for biomarker analysis set of EUT.

9.3.3. Evaluation of Changes in Selected Laboratory Parameter

The analysis of relevant changes in selected laboratory parameter will be described in Section 10.4.

9.3.4. Evaluation of Pharmacokinetics of Treprostinil Palmitil

The analysis of TP plasma PK parameter of C_{max}, t_{max}, $AUC_{t1–t2}$, $AUC_{0–inf}$, $t_{1/2,}$ CL/F and Vd/F will be described in Section 11.

9.3.5. Evaluation of Respiratory Gas Exchange

Respiratory gas exchange parameter of minute ventilation, VCO_2, $ETCO_2$, SpO_2 (from respiratory gas exchange), oxygen pulse, oxygen consumption, heart rate, minute ventilation-CO_2 production ratio (VE/VCO_2) and its changes over 24 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) and presented in a by-participant listing for the safety population.

9.3.6. Evaluation of TPIP on Parameters of Right Ventricular Function

Observed values of LVEF, TVR maximum velocity, TAPSE, and end diastolic right ventricular volume (indexed to BSA) and their changes from baseline at 2 to 8 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) and presented in a by-participant listing for the safety population.

9.3.7. Evaluation of TPIP on Pulmonary Vasculature and Blood Flow Parameters

Pulmonary vasculature and blood flow parameters observed by a pulmonary CT scan for total blood volume, blood volume in vessels < $5mm^2$ and their changes from baseline at 4 to 8 hours after TPIP administration in single-dose inpatient treatment period will be summarized by dose group (if applicable) and presented in a by-participant listing for the safety population.

9.3.8. Evaluation of TPIP on Exercise Capacity (6MWD), Extended Use Treatment Only

Observed values for 6MWD and the change in 6MWD from Baseline EUT to EUT Week 16 will be summarized by dose group (if applicable) for the safety population. A by-participant listing, using the same analysis population, will present the observed

values of single-dose inpatient treatment period and all observed values and its changes of EUT.

10. SAFETY

Unless otherwise stated, the safety population will be used for summaries and listings of safety variables.

10.1. Exposure to Study Drug and Compliance

As there is only one study drug administration in single-dose inpatient treatment period under direct observation of site personnel, an administration of study drug as planned will be assumed for this period.

The total exposure to study drug in EUT period will be calculated. The duration of EUT period will be calculated as described in Section 7.3.1. The start date duration of exposure in EUT will be the date of first dispensed study drug in EUT, the end date of EUT for duration of exposure will be the date of last study drug returned in EUT or the date of discontinuation of EUT (whatever occurs first).Compliance to intake of study drug will not be calculated and listed.

The exposure will be presented in a by-participant listing (study drug dosing date/time, date/time of discharge from hospital in single-dose inpatient treatment period, length of EUT period, total dose in EUT period) for the safety population.

10.2. Adverse Events

An AE is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. All AEs will be captured on the eCRF. The AE verbatim descriptions (investigator terms from the eCRF) will be classified into standardized medical terminology using MedDRA. AEs will be coded to primary SOC and PT using MedDRA, Version 25.0 or higher.

TEAEs are those AEs that occurred on or after the date/time of first study drug dosing and up to (and including) Study Day 30 follow-up visit or Study Day 30 follow-up of EUT for participants attending the EUT.

Where AE start dates are missing or partially missing, AEs will be assumed to be treatment-emergent, except if the partial start dates or the AE end date indicate that the AE started before the study drug dosing (see

Table 2).

Table 2: TEAE Assignment in Case of Missing AE Start Date Elements

Missing elements of AE start	Rule	
Regardless of any missing information for AE start: AE end date < first study drug dosing date		non-TEAE
Otherwise (i.e., if AE end date ≥ first study drug dosing date)		
- all		TEAE
- day and month	AE start year ≥ first study drug dosing year	TEAE
	AE start year < first study drug dosing year	non-TEAE
- day	AE start month / year ≥ first study drug dosing month / year	TEAE
	AE start month / year < first study drug dosing month / year	non-TEAE

Duration of AEs will be calculated in days (see Section 7.3.1). If date of onset or end date of event is not complete, duration will be set to missing.

An AE overview table for overall treatment group (if applicable also by dose group) will include number and percentage of participants and number of events with:

- AE;
- TEAE;
- Maximum severity of TEAE by participant;
- Treatment-related TEAE;
- Serious TEAE;
- Treatment-related Serious TEAE;
- TEAE leading to withdrawal of study drug, based on end of treatment eCRF;
- TEAE leading to withdrawal from study, based on end of study eCRF;
- TEAE resulting in death;
- AE related to COVID-19;
- TEAE related to COVID-19.

The overview table will be given for the whole study period, single-dose inpatient treatment period and EUT. The start of EUT will be the date of EUT Day 1.

A frequency table for overall treatment group (if applicable also by dose group) displaying TEAEs by SOC and PT will be provided. The summary will be ordered by descending number of participants with that SOC, the same is PT within each SOC. If more than one SOC is affected by the same number of participants, those SOC will be ordered alphabetically, the same is for PTs with the same SOC. The summary table will be given for the whole study period, single-dose inpatient treatment period and EUT.

All AEs . The listing will include all data collected for AEs, including a flag indicating treatment-emergency, onset day in relation to study drug dosing, duration of AE, and assignment of AE to single-dose inpatient treatment period or EUT.

10.3. Deaths

A by-participant listing of all AEs resulting in death will be provided.

10.4. Clinical Laboratory Evaluation

Laboratory test results and their changes from baseline (for numerical outcomes, if applicable) for hematology, clinical chemistry, coagulation, and serology tests will be summarized by time point.

All laboratory results and changes from baseline will be presented in a by-participant listing.

Table 3: Protocol-Required Safety Laboratory Tests

Category	Laboratory Parameters
Hematology	Hemoglobin, erythrocytes, hematocrit, MCH, MCV, MCHC, leukocytes, differential blood count of neutrophils, eosinophils, basophils, monocytes, lymphocytes, and platelets
Clinical Chemistry	Sodium, chloride, potassium, CO_2, magnesium, calcium, glucose, phosphate, total bilirubin (with direct and indirect fractionation, if total bilirubin is elevated >2 ULN), alkaline phosphatase, LDH, AST, ALT, CPK, albumin, total protein, creatinine, urea-nitrogen, uric acid, and estimated glomerular filtration rate
Coagulation	Prothrombin time, PTT, INR
Serology	HIV antibody, HBsAg, hepatitis C virus antibody

ALT = alanine aminotransferase; AST = aspartate aminotransferase; CO_2 = carbon dioxide; CPK = creatine phosphokinase; hCG = human chorionic gonadotropin; HIV = human immunodeficiency virus; HBsAg = hepatitis B surface antigen; INR = international normalized ratio; LDH = lactate dehydrogenase; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; PTT = partial thromboplastin time; ULN = upper limit of normal

10.5. Vital Signs

Heart rate, respiratory rate, systolic and diastolic blood pressure, body temperature, mean arterial pressure, and oxygen saturation will be collected at protocol specified visits and time points in single-dose inpatient treatment period and EUT. Measured values together with changes from baseline will be summarized by descriptive statistics by time point and by dose group (if applicable) and presented in a by-participant listing.

A separate summary (if applicable) and listing will be provided for safety analysis set of EUT.

10.6. Physical Examination

Any abnormalities noticed by investigator during the physical examination at screening will be recorded in the medical history, or as an AE if occurred or worsens after ICF was signed.

Dates of physical examination will not be listed.

10.7. Electrocardiograms

The results of overall investigator 12-lead ECG interpretation will be summarized by time point and by dose group (if applicable) and presented in a by-participant listing.

10.8. Pregnancy

Pregnancy results will be listed for all participants with outcome.

11. PHARMACOKINETIC ANALYSIS

11.1. Pharmacokinetic Sample Analysis

PK samples will be analyzed using a validated liquid chromatography coupled with tandem mass spectrometry assay for TRE and TP in human plasma. Assay results and validation details will be provided in a separate bioanalytical report.

11.2. Pharmacokinetic Sampling

Blood samples for PK analysis of TRE and TP will be collected at pre-dose, 30min, 60min, 2, 4, 8, 24, and 48h after dosing with TPIP in single-dose inpatient treatment period.

For the PK sampling, a window of ± 10min will be allowed for sample collection at 30min, 60min, at 2h a window of ± 20min, at 4h a window of ± 1h, at 8 and 24h a window of ± 2h, and a window of ± 4h will be allowed at 48h planned sampling time.

Samples that are collected outside these windows will be logged as protocol deviations, but it is anticipated that PK data will still be used for PK parameter derivation.

11.3. Below the Limit of Quantification Values / Missing Values

For PK parameter calculations, pre-dose below the limit of quantification (BLQ) data will be treated as zero. All post-dose values BLQ will be considered as “missing”. For descriptive statistical summaries and graphing, BLQ data will be considered as zero.

11.4. Pharmacokinetic Parameters

PK parameters will be derived from plasma concentrations of TRE and TP using noncompartmental methods with Phoenix WinNonlin (Certara USA Inc., Princeton, New Jersey) Version 8.4 or higher. This derivation will be done at Parexel. The following plasma PK parameters will be calculated as endpoints for TRE and TP using actual sampling times, as shown in Table 4.

Table 4: PK Parameters to be determined

Parameter	Description
C_{max}	Maximum observed plasma concentration of study drug, obtained directly from the concentration-time data
t_{max}	Time to maximum observed plasma concentration, obtained directly from the concentration-time data
AUC_{0-t}	Area under the plasma concentration versus time curve (AUC) from time 0 to the last quantifiable concentration, calculated using linear- up/log-down method
AUC_{0-inf}	AUC from time 0 extrapolated to infinity
$t_{1/2}$	Elimination half-life which will be estimated by linear regression of concentration versus time data presented in a log-linear scale. A minimum of 3 data points in the elimination phase, not including C_{max}, will be used for the calculation. The λz will not be estimated if r-squared is less than 0.8.
CL/F	Apparent total clearance of study drug from plasma after extravascular administration, calculated from; $CL/F = \frac{Dose}{AUC_{0-inf}}$
Vd/F	Apparent volume of distribution at terminal phase, calculated from; $CL/F/\lambda_z$

The PK parameters for each participant will be determined as data permit and as appropriate.

11.5. Pharmacokinetic Statistical Analysis

Plasma concentrations of TRE and TP will be presented in a by-participant listing for the PK analysis set.

Plasma concentration versus time profiles based on actual sampling times for each participant will be presented graphically at linear and log scale.

PK parameters for TRE and TP will be presented in a by-participant listing for the PK analysis set.

12. CHANGES TO PROTOCOL-SPECIFIED ANALYSES

Data for ETO_2, as a parameter of respiratory gas exchange, are not covered by eCRF nor available in any other data source. Therefore those data will be not part of any summary nor at any by-participant listing.

13. REFERENCES

1) International Council for Harmonisation (ICH) E6 (R2) GCP: Integrated Addendum to ICH E6 (R1) Guidance for Industry, published in the Federal Register (83 Federal Register 8882 [2018]) (https://www.fda.gov/media/93884/download.)
2) Protocol INS1009-201: A Phase 2a, An Open-Label Single Dose Study to Assess the Safety, Pharmacokinetics, and Pharmacodynamics of Treprostinil Palmitil Inhalation Powder in Participants with Pulmonary Arterial Hypertension, Version 1.0, 02 Nov 2020
3) Protocol INS1009-201: A Phase 2a, An Open-Label Single Dose Study to Assess the Safety, Pharmacokinetics, and Pharmacodynamics of Treprostinil Palmitil Inhalation Powder in Participants with Pulmonary Arterial Hypertension, Version 2.0, Amendment 1, 30 Sep 2021

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