Official Title: A Phase 1, Open-Label, Non-Randomized, Parallel-Group, Multiple-Escalating -Dose Pharmacokinetic Study of Nalbuphine Extended-Release Oral Tablets in Subjects with Impaired Hepatic Function Compared to Healthy Subjects and Exploratory Effect on Itch

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Statistical Analysis Plan

A PHASE 1, OPEN-LABEL, NON-RANDOMIZED, PARALLEL-GROUP, MULTIPLE-ESCALATING-DOSE PHARMACOKINETIC STUDY OF NALBUPHINE EXTENDED-RELEASE ORAL TABLETS IN SUBJECTS WITH IMPAIRED HEPATIC FUNCTION COMPARED TO HEALTHY SUBJECTS AND EXPLORATORY EFFECT ON ITCH

Sponsor Study No. TR10

inVentiv Health Clinique inc. Project No. 182018

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inVentiv Project No.: 182018

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LIST OF ABBREVIATIONS

AE	Adverse event
AUC_{0-12}	Area under the concentration-time curve from time zero to 12 hours
AUC_{0-24}	Area under the concentration-time curve from time zero to 24 hours
AUC_{0-inf}	Area under the concentration-time curve from time zero to infinity
AUC_{0-t}	Area under the concentration-time curve from time zero to last measurable concentration
BLQ	Below the lower limit of quantitation
BMI	Body mass index
CI	Confidence interval
CL/F	Total body clearance
C_{max}	Maximum observed plasma concentration
CSR	Clinical study report
C_{trough}	The concentration at the end of the dosing interval
CV	Coefficient of variation (equivalent to C.V.)
Df	Degree of freedom
Fl (%)	Percentage of fluctuation
ECG	Electrocardiogram
g	Gram
ICF	Informed consent form
Inc.	Incorporated
K_{el}	Terminal elimination rate constant (equivalent to λz)
$K_{el\ Lower}$	The time point where K_{el} calculation begins
$K_{el\ Upper}$	The actual sampling time of the last measurable concentration used to estimate the K_{el}
Ln	Natural logarithm (equivalent to ln)
MAD	Multiple ascending dose
Max	Maximum (equivalent to max.)
MedDRA®	Medical Dictionary for Regulatory Activities
mg	Milligram
Min	Minimum (equivalent to min.)
mL	Milliliter
n	Number of observations
No.	Number

PK	Pharmacokinetic
PD	Pharamcodynamic
PT	Preferred Term
QRS	The QRS complex is a structure on the ECG that corresponds to the depolarization of the ventricles
QT	Time from electrocardiogram Q wave to the end of the T wave corresponding to electrical systole
QTcF	QT corrected with Fridericia formula (msec) = QT (msec) / $RR^{1/3}$ (sec)
RR	Duration of ventricular cardiac cycle
SAD	Single ascending dose
SAE	Serious adverse event
SAP	Statistical analysis plan
SAS®	Statistical analysis system
SD	Standard deviation
SOC	System Organ Class
SOP	Standard Operating Procedure
TEAE	Treatment-emergent adverse event
$T_{½\ el}$	Terminal elimination half-life
T_{max}	Time of maximum observed plasma concentration
vs	Versus
V_d/F	Volume of distribution
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 inVentiv. Analyses specified in this plan are based on Trevi Therapeutics, Inc. Study No. TR10 Final Version 3.0: dated 05 Nov, 2019 (inVentiv Project No. 182018).

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 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 which 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 inVentiv's Standard Operating Procedures (SOPs). 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

2.1 Primary Objectives

- To evaluate the effect of hepatic impairment on nalbuphine extended release (NAL ER) pharmacokinetics (PK) as a function of dose.
- To evaluate the safety and tolerability of NAL ER in hepatic impaired subjects.

2.2 Secondary Objectives

- To select doses and dosing frequency for NAL ER tablets in subjects with impaired hepatic function.
- To assess potential of oral NAL ER to reduce itch in the hepatic impaired population

3. Study Design

3.1 General Design

This is a Phase 1, open-label PK and safety study of NAL ER in subjects with impaired hepatic function compared to healthy subjects. It will be performed in 2 parts:

- Part 1: single-ascending-dose (SAD) cohorts
- Part 2: multiple-ascending-dose (MAD) cohort

Part 1 (SAD) – Dose Cohorts 1 to 5

- Cohort 1 (27 mg): 6-8 subjects with mild hepatic impairment, 6-8 subjects with moderate hepatic impairment, and 4-6 subjects with severe hepatic impairment
- Cohort 2 (54 mg): 6-8 subjects with mild hepatic impairment and 6-8 subjects with moderate hepatic impairment
- Cohort 3 (108 mg): 6-8 subjects with mild hepatic impairment and 6-8 subjects with moderate hepatic impairment
- Cohort 4 (162 mg): 6-8 subjects with mild hepatic impairment and 6-8 subjects with moderate hepatic impairment
- Cohort 5 (highest dose tested in subjects with mild or moderate hepatic impairment): 6-8 healthy control subjects who have been appropriately age-, body mass index (BMI), and gender-matched to subjects with mild and moderate hepatic impairment from Cohorts 1 to 4

In Part 1 of the study (SAD), each of the cohorts will be dosed sequentially starting with the lowest dose for subjects with mild or moderate hepatic impairment. Subjects enrolled in Cohort 1 can also be enrolled in Cohorts 2, 3, and 4.

For each dose cohort, enrollment of subjects with mild (Group 1) or moderate (Group 2) hepatic impairment can be done in parallel. An evaluation of safety and tolerability of the combined mild and moderate hepatic impairment Group 1 and Group 2 subject data will be done at each dose level before proceeding to the next dose level.

Subjects with severe impairment (Group 3) will be enrolled with the 27 mg dose upon completion of SAD in subjects with mild and moderate impairment. After review of the safety and tolerability for the first 2 severe subjects dosed at 27 mg it will be determined whether or not to complete that dose level for the remaining severe subjects.

At the completion of all subjects dosed in the cohort, there will be an evaluation of safety and tolerability to determine whether to proceed to the next dose level for this group.

The drug pharmacokinetics in the hepatic impairment subject population will be compared relative to the healthy subject population (Cohort 5).

Part 2 MAD – Dose Cohort 6

- Cohort 6: 6-8 subjects with mild and moderate hepatic impairment

In Part 2 of the study (MAD), subjects will receive multiple doses over 13 days according to a dose escalation scheme. Part 2 of the study may be initiated after Groups 1 and 2 (which consist of subjects with mild and moderate hepatic impairment) complete Part 1 of the study (SAD) and following satisfactory review of the safety and tolerability data by the Safety Committee.

Subjects enrolled in Part 1 can also be enrolled in Part 2. However, once the Part 1 SAD portion of the study is completed, if in the judgement of the Safety Committee the PK data demonstrates dose linearity across the dose range in both the mild and moderate hepatic impairment subjects (Group 1 and Group 2 subjects), such that PK modeling can be predictive of steady state dosing levels, a decision may be made to end the study without conducting Part 2 of the study

3.2 Subject Distribution:

Cohorts 1 to 4 (Part 1, SAD) and 6 (Part 2, MAD) will each include the following groups of hepatic-impaired subjects:

- Group 1: Child-Pugh Group A: score 5-6 points; 6-8 subjects with mild hepatic impairment
- Group 2: Child-Pugh Group B: score 7-9 points; 6-8 subjects with moderate hepatic impairment
- Group 3 : Child-Pugh Group C: score 10-15 points; 4-6 subjects with severe hepatic impairment
- Cohort 5 (Part 1, SAD) will include 6-8 demographically matching healthy subjects with normal hepatic impairment (control group).

3.3 Study Procedures

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

3.4 Treatment Description

The treatments administered in this study are presented in Table 3-1 and Table 3-2.

Table 3-1 Treatment Description for Part 1 (SAD)

Part 1 – Single Ascending Dose with 5 cohorts	
Cohort	**Planned dose of NAL ER**
1	27 mg
2	54 mg
3	108 mg
4	162 mg
5 (healthy subjects)	Up to 162 mg

For Cohort1, a minimum of 6 subjects from Group 1 (mild impairment) and 6 subjects from Group 2 (moderate impairment), and 4 subjects from Group 3 (severe impairment) will receive a single dose of NAL ER.

For Cohorts 2-5, a minimum of 6 subjects from Group 1 (mild impairment) and 6 subjects from Group 2 (moderate impairment) will receive a single dose of NAL ER. Cohort 5 (healthy) will also receive a single dose of NAL ER as determined by the Safety Committee review of the mild and moderate impaired data.

Cohorts 1-4 will be dosed sequentially in an ascending design. Enrollment of subjects with mild impairment and moderate impairment can be done in parallel. Subjects with severe impairment will be enrolled upon completion of the highest dose level in mild and moderate impairment subjects. There will be at least 7 days between dosing of each dose level, within the same group of impairment (mild and moderate). Subjects will be observed for a period of 4 days.

Safety, tolerability, and combined mild and moderate impaired subject data will be evaluated by a Safety Committee at the completion of each cohort dose level before proceeding to the next cohort dose level. Therefore, the highest dose administered may be lower than 162 mg. Depending on safety and tolerability, the dose escalation scheme may be modified such that intermediate dose levels are administered.

Based on the overall safety and tolerability observed in the subjects with mild and moderate impairment, at the completion of all doses tested, it will be determined by the Safety Committee whether or not it is safe to proceed with dosing for the first 2 subjects with severe impairment starting with the 27 mg dose. After review of the safety and tolerability for the first 2 severe

subjects dosed, it will be determined whether or not to complete that dose level for the remaining severe subjects at the 27 mg dose

At the completion of all subjects dosed in the cohort, there will be an evaluation of safety and tolerability to determine whether to proceed to the next dose level for this group.

Table 3-2 Treatment Description for Part 2 (MAD)

Part 2 – Multiple Ascending Dose for Cohort 6		
Study Day	**NAL ER Dose (mg)**	
	AM	PM
1	27	0
2	27	27
3	27	27
4	27	54
5	54	54
6	54	54
7	54	108
8	108	108
9	108	108
10	108	162
11	162	162
12	162	162
13	162	-

Drug administration in Part 2 of the study (MAD) may be initiated following satisfactory review of the safety and tolerability data from Groups 1 and 2 (mild and moderate impairment) from Part 1 of the study (SAD) by the Safety Committee. Depending on safety and tolerability, the dose escalation scheme may be modified such that intermediate dose levels are administered. The dosage regimen (once daily versus twice daily) will be confirmed following review of PK data from Part 1 of the study (SAD). Part 1 subjects may enter Part 2 of the study after a 7 day washout period.

3.5 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 delegate may withdraw any subject from the study for one of the reasons described below; subject withdrawal will be done in accordance with SOPs of the clinical sites:

- Safety reason;
- Non-compliance with protocol requirements;
- Significant protocol deviation;
- Positive urine alcohol or drug screen, or pregnancy test.

Subjects missing one dose of scheduled IP or experiencing emesis during the study will be evaluated on a case-by-case basis for possible impact on the outcome of the study.

Hematology, clinical chemistry, urinalysis, and coagulation results will be reviewed by an Investigator prior to first dosing; 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. For subjects with hepatic impairment and taking medications that are allowed by the Investigator, a positive result to opiate/benzodiazepine/THC screen (drug screen) may have derived from these medications and these subjects will not be automatically withdrawn from the study. Subjects who withdraw or are withdrawn from the study after dosing, for reasons other than safety and tolerability, may be replaced after consultation between the Investigator and Sponsor. The total number of subjects dosed (including potential replacement subjects) will remain within a maximum of 112 subjects for the whole study.

3.6 Dose Escalation Stopping Rules

Dose escalation can be stopped if any treatment-emergent adverse event (TEAE) occurs in a subject dosed with NAL ER during the study that, in the opinion of the Investigator, raises concerns about the safety and tolerability of a higher dose. In addition, the following dose escalation rules will apply.

Dose-limiting toxicity (DLT) in the hepatic-impaired subject population participating in this study is defined as the dose at which a confirmed Grade 3 adverse event develops in any 2 subjects even if in two different organ systems, that is IP related and is unlikely to be related to the disease process. Overnight oximetry that drops more than 10% from a subject's baseline lowest value for a consistent period of 5 minutes will be considered a DLT TEAE.

Any subject who experiences a DLT TEAE will not receive further dose of study drug, and safety follow-up procedures will be performed.

4. Changes from the Protocol

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

5. Analysis Populations

5.1 Safety Population

The safety population is defined as all subjects who received the Investigational Product (IP).

5.2 Pharmacokinetic Population

The PK population will include all subjects completing one dose level of the study and for whom the PK profile can be adequately characterized. This Analysis will be conducted if warranted and reported separately. Subjects missing one dose of scheduled IP or experiencing emesis during the study will be evaluated on a case-by-case basis for possible impact on the outcome of the study.

6. Interim Analyses

Review of interim safety analyses will be performed between cohorts or groups. Interim PK analyses may be performed between cohorts or groups.

The PK parameters will be calculated using scheduled sampling times as actual sampling times will not be available for these preliminary analyses.

6.1 Subject Disposition

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

6.2 Protocol Deviations

The protocol deviations will be categorized and listed by subject.

6.3 Demographics and Baseline Characteristics

The descriptive statistics (mean, median, standard deviation [SD], minimum [Min], maximum [Max], and sample size as n) will be calculated for continuous variables (age, BMI, height, and weight) considering last results (scheduled or unscheduled) obtained prior to the first dose administration. Frequency counts and percentages will be tabulated for categorical variables (age group, gender, ethnicity, and race). Results will be presented by part, cohort and overall for each study population. All demographic characteristics will be listed by subject.

6.4 Medical History

Medical history will be listed by subject. The Medical Dictionary for Regulatory Activities (MedDRA®) Version 22.0 will be used to classify all medical history findings by System Organ Class (SOC) and Preferred Term (PT).

6.5 Prior and Concomitant Medications

The use of concomitant medications will be monitored throughout the study. The World Health Organization Drug Dictionary (WHO DD) Version Jun2018, format B will be used to classify all medication reported during the study.

Prior and concomitant medications will be listed by subject.

6.6 Study Drug Administration

The study drug administration details including treatment or dose received (nalbuphine) and date and time of administration will be listed by subject for each study part.

7. Safety Analyses

Safety data will be evaluated through the assessment of AEs (i.e., seriousness, severity, relationship to the study drug, outcome, duration, and management), laboratory evaluations, 12-lead electrocardiogram (ECG), clinical signs and symptoms from physical examination, vital signs, and pulse oximetry assessments. TEAEs, laboratory values, vital signs, and ECGs will be summarized by cohort/treatment and time point of collection, according to the group and/or overall, as appropriate. Safety data will be summarized but will not be subjected to inferential analysis.

7.1 Physical Examination Findings

For Part 1 and Part 2 of the study, complete physical examinations will be performed at screening. The complete physical examination includes at least the following components: HEENT (head, eyes, ears, nose, and throat), neck, and lungs, cardiovascular, abdomen, skin, musculoskeletal evaluation, and general neurological examination.

For Part 1, a brief physical examination will be performed on Day -1 and Day 4.

For Part 2, a brief physical examination will be performed on Day -1 and Day 16.

Any abnormal findings judged to be clinically significant will be documented as medical history or as an AE, depending upon time of observation, as appropriate. Any physical examination findings documented as AEs will be included in the AE summaries.

Body measurements will be performed at screening and will include: body weight, height measurement, and BMI. Body measurement will be summarized (mean, median, SD, Min, Max, and sample size) in demographic tables (safety and PK).

7.2 Adverse Events

Treatment-emergent AEs (TEAEs) and non-TEAEs will be listed. TEAEs will be defined as AEs that occur on or after the date and time of study drug administration. Any AE that first occurs pre-dose but worsens in severity after the first study drug administration will also be considered a TEAE. Non-TEAEs are those that occur prior to the first administration of the study medication and resolved prior to dosing or that first occur prior to the first study drug administration but do not worsen in severity after dosing. TEAEs will be captured during the study until study exit. Adverse events will be followed-up until complete resolution, or until the Principal Investigator or Medical Sub-Investigator judges safe to discontinue follow-up.

The incidence of TEAEs will be summarized for each study part separately using the safety population. The MedDRA® dictionary Version 22.0 will be used to classify all TEAEs reported during the study by SOC and PT.

Incidence of subjects who experienced TEAEs will be presented by treatment, group 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.

For each recorded AE or SAE, the Investigator or designee must make an assessment of intensity, if possible, based on the Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 or later (*https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf).* A determination of grading at the time of AE occurrence is required in order to monitor for DLTs and may be modified upon continued assessment. If there is insufficient information to determine intensity, the AE must still be reported. The severity of an AE that cannot be termed and graded by the most current version of CTCAE will be categorized as follows (Table 4):

Table 4: Classification of Adverse Event Intensity

Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
Grade 2	Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living.
Grade 3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living.
Grade 4	Life-threatening consequences; urgent intervention indicated.
Grade 5	Death.

The relationship of TEAEs will be classified according to the study protocol as unrelated (not related), unlikely, possible, probable to nalbuphine.
The outcome of TEAEs will be classified according to the study protocol as fatal, resolved, resolved with sequelae and unresolved to nalbuphine.

Incidence of subjects who experienced TEAEs (frequency and the percentage of subjects) will be presented for each group and overall by:

- SOC and PT;
- SOC, PT, and relationship;

- SOC, PT, and maximum severity.

Number of TEAEs will also be presented by:

- SOC and PT;
- SOC, PT, and relationship;
- SOC, PT, and maximum severity.

Serious adverse events (SAEs) and TEAEs leading to early discontinuation of study drug will be listed separately.

7.3 Laboratory Parameters

For Part 1 of the study, clinical chemistry will be performed at screening, in the morning on Day -1, and on Day 4. For Part 2 of the study, clinical chemistry will be performed at screening, in the morning on Day -1, and on Days 5, 10, and 16. The following will be assessed: albumin, alkaline phosphatase, AST, ALT, urea, calcium, chloride, glucose, phosphorus, potassium, creatinine, sodium, bicarbonate (CO2), total bilirubin, indirect bilirubin, CPK, direct bilirubin, and total protein.

Considering that indirect bilirubin is calculated from total and direct bilirubin values, indirect bilirubin result would not be available in case of direct bilirubin below the limit of quantification.

Estimated glomerular filtration rate (eGFR) will be calculated by the MDRD4 Equation at screening.

For Part 1 of the study, hematology will be performed at screening, in the morning on Day -1, and on Day 4. For Part 2 of the study, hematology will be performed at screening, in the morning on Day -1, and on Days 5, 10, and 16. The following will be assessed: complete blood count with differential, hemoglobin, and hematocrit.

For Part 1 of the study, coagulation tests (PT, aPTT, and INR) will be performed at screening, in the morning on Day -1, and on Day 4. For Part 2 of the study, coagulation tests will be performed at screening, in the morning on Day -1, and on Day 16. The following will be assessed: prothrombin time/international normalized ratio (PT/INR) and activated partial thromboplastin time (aPTT).

For Part 1 of the study, urinalysis will be performed at screening, in the morning on Day -1, and on Day 4. For Part 2 of the study, urinalysis will be performed at screening, in the morning on Day -1, and on Day 16. 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 on abnormal findings and these results will be listed only.

Listings of all clinical laboratory results (Clinical Chemistry, Hematology, Urinalysis and Coagulation) will be provided with the abnormal values flagged with "L" for low and "H" for high for continuous parameters, and “A” for abnormal for categorical parameters.

Descriptive statistics (mean, median, SD, Min, Max, and sample size) for each clinical laboratory test (continuous variables) will be presented by Cohorts and group for screening, check-in on Day -1 and study exit. Change from baseline descriptive statistics for study exit will be presented. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to the administration of nalbuphine. For categorical variables (urinalysis tests), the number of subjects (frequency and percentage) will be tabulated for each individual result (e.g., negative, positive, trace). Results from unscheduled or 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, or for baseline determination if applicable.

A summary table of shifts from baseline to study exit measurements will be provided. Baseline will be defined in the same manner as described in the preceding paragraph for continuous variables. The shift tables will include normal, low, and high relative to the laboratory reference ranges (or normal-abnormal for categorical variables). 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.

If more than one clinical laboratory is used for the study, a formula that takes into consideration the relative normal ranges of each test of laboratories used will be applied in order to normalize these data. The conversion formula used will depend on the typical distribution of the normal range for each laboratory test; the two formulae used are presented below:

- Hemoglobin, hematocrit, and platelet count test results are considered to have a normal distribution (Chuang-Stein, 1992) and the following formula will be used (Karvanen J., 2003):

$$s = L_s + (x - L_x)\frac{U_s - L_s}{U_x - L_x}$$

- The remaining hematology, biochemistry, and urinalysis test results are considered to have a non-normal distribution (Chuang-Stein, 1992) and the following formula will be used (Karvanen J., 2003):

$$s = \frac{x\,U_s}{U_x}$$

U= upper limit; L= lower limit; s= primary facility result; and x= secondary facility results.

Prior to applying these formulae, if required, units will be adjusted.

7.4 Vital Signs

Blood pressure, heart rate, and respiratory rate will be measured in a sitting position after at least 5 minutes of sitting (except for safety reasons).

In Part 1 of the study (SAD), blood pressure, heart rate, and respiratory rate will be measured at screening, in the morning on Day -1, pre-dose, approximately 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose, and on Day 4. Body temperature will be measured at screening, Day -1, and Day 4. In Part 2 of the study (MAD), blood pressure, heart rate, and respiratory rate will be measured screening, in the morning on Day -1, pre-morning dose every day (Days 1 to 13), approximately 1, 2, 4, 6, 8, and 12 hours post-morning dose on Day 1 and Day 13, approximately 2 and 6 hours post-morning dose on Days 2 to 12, and once on Days 14, 15, and 16. Body temperature will be measured at screening, Day -1, and Day 16.

When vital signs measurements coincide with a blood draw, they should preferably be performed before the blood collection whenever possible. A time window of 15-20 minutes for vital signs will be allowed if there are multiple procedures at the same time point.

A listing of all vital signs results will be provided.

Descriptive statistics (mean, median, SD, Min, Max, and sample size "n") for each vital sign measurement will be presented considering each study part separately, by cohort and group for each time point. Change from baseline descriptive statistics for all post-dose time points will also be presented. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to drug administration in each part/cohort.

Results from unscheduled or 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, or for baseline determination if applicable.

7.5 Pulse Oximetry

Pulse oximetry will be performed at the screening visit. Baseline continuous pulse oximetry will be performed during the nighttime of Day -1 (minimum of 8 hours prior to first dose administration).

For Part 1 of the study (SAD), following the dose and throughout study conduct, oxygen saturation will be measured via pulse oximetry at pre-dose and 1.5, 4.5, and 8 hours post-dose. Oxygen saturation will be monitored via continuous pulse oximetry overnight beginning at bedtime and until awaking the next morning from Day 1 to end of confinement with oxygen saturation measurements specifically recorded at approximately midnight and 4 am.

For Part 2 of the study (MAD), following the first dose and throughout study conduct, oxygen saturation will be measured via pulse oximetry at pre-morning dose, at 1.5, 4.5, and 8 hours following the morning dose, and at 2 hours following the evening dose. Oxygen saturation will be

monitored via continuous pulse oximetry beginning at bedtime and until awaking the next morning from Day 1 to end of confinement with oxygen saturation measurements specifically recorded at approximately midnight and 4 am.

Pulse oximetry measurements may be collected within 10 minutes before or after the specified time point.

A listing of all Pulse oximetry results will be provided.

7.6 12-lead Electrocardiogram

For Part 1 of the study (SAD), a 12-lead ECG will be performed at screening, in the morning on Day -1, pre-dose, approximately 1, 2, 4, 6, 8, 12, 24, and 48 hours post-dose, and on Day 4.

For Part 2 of the study (MAD), a 12-lead ECG will be performed at screening, in the morning on Day -1, pre-morning dose every day (Days 1 to 13), approximately 1, 2, 4, 6, 8, and 12 hours post-morning dose on Day 1 and Day 13, and once on Days 14, 15, and 16.

ECG will be performed after a resting time of at least 5 minutes in supine position. When ECG coincides with a blood draw, it should preferably be performed before the blood collection whenever possible. A time window of 15-20 minutes for ECGs will be allowed if there are multiple procedures at the same time point.

A listing of all ECG results will be provided.

Descriptive statistics (mean, median, SD, Min, Max, and sample size “n”) for each measurement (continuous variables) will be presented considering each study part separately, by cohort and group for each time point. Change from baseline descriptive statistics all post-dose time points will also be presented. Baseline will be defined as the last results (scheduled or unscheduled) obtained prior to drug administration in each part and cohort. Results from unscheduled or 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, or for baseline determination if applicable.

8. Pharmacokinetic Analyses

8.1 General PK Considerations

- All samples from nalbuphine-dosed subjects will be analyzed and all concentrations listed.
- The listing of PK concentrations will be flagged for subjects who did not receive all doses and any other significant protocol deviations.
- Subjects missing one dose of scheduled IP or experiencing emesis during the study will be evaluated on a case-by-case basis for possible impact on the outcome of the study.
- Descriptive statistics will be performed for all time points available, with the exclusion of subjects who did not receive all doses or any other significant protocol deviations.
- Pharmacokinetic parameters will be derived where possible for all subjects. Data from subjects with incomplete profiles (missed blood draws, lost samples, samples unable to be quantified) may be used if PK parameters can be estimated using the remaining data points.
- Descriptive statistics will be performed on all parameters available, and any missing parameters will be flagged.

8.2 Handling of the BLQ and the No Reportable Concentration Values

All concentration values below the lower limit of quantitation (BLQ) and samples with no reportable value occurring prior to the first dosing will be replaced by "0.00". For tabulation, graphical representation and calculation purposes, all samples with no reportable value observed after administration of the first dosing will be set to missing.

8.3 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. 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. In the PK section of the report, scheduled sampling times will be presented in concentration tables and mean graphs while actual times will be presented for the individual graphs. A listing of the actual times will be provided for PK samples.

8.4 Pharmacokinetic Concentration and Parameters

8.4.1 Part 1 (SAD) - Cohort 1 to 5

A total of 11 blood samples will be drawn for quantification of nalbuphine and its metabolites (if required) from each subject for PK analyses. Blood samples will be collected prior to drug administration and at 1.50, 3.00, 5.00, 7.00, 9.00, 12.0, 24.0, 36.0, 48.0, and 72.0 hours post-dose.

Plasma concentrations from nalbuphine and its metabolites (if required) will be used to calculate the following parameters by standard non-compartmental methods:

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, calculated as $AUC_{0-t}+C_t/K_{el}$, where: C_t = the last measurable concentration.
C_{max}:	Maximum observed concentration.
T_{max}:	Time of observed C_{max}.
$T_{½\ el}$:	Terminal elimination half-life, calculated as $ln(2)/K_{el}$.
Residual area	Calculated as $100*(1- AUC_{0-t} / AUC_{0-inf})$.
Cl/F:	Apparent total body clearance, calculated as $Dose/AUC_{0-inf}$ for nalbuphine.
V_d/F:	Apparent volume of distribution during terminal phase, calculated as $Dose/(K_{el} \times AUC_{0-inf})$ for nalbuphine.
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. At least 3 concentration points will be used in estimating K_{el}. C_{max} should not be used in this estimation unless there are only two other quantifiable concentrations available following the C_{max}. The time point where log-linear K_{el} calculation begins ($K_{el\ Lower}$), and the actual sampling time of the last quantifiable concentration used to estimate the K_{el} ($K_{el\ Upper}$) will be reported with the correlation coefficient from the linear regression to calculate K_{el}.

8.4.2 Part 2 (MAD) – Cohort 6

On Day 1 a total of 10 blood samples will be drawn for quantification of nalbuphine and its metabolites in plasma from each subject for PK analyses as follows:

- At Day 1 – Pre-dose and at 1.50, 3.00, 4.00, 5.00, 6.00, 7.00, 9.00, 12.0, and 24.0 hours post-dose.

On Days 4, 7 and 10 a total of 9 blood samples will be drawn for quantification of nalbuphine and its metabolites in plasma from each subject for PK analyses as follows:

- At Day 4, 7 and 10 – pre-morning dose and at 1.50, 3.00, 4.00, 5.00, 6.00, 7.00, 9.00, and 12.0 hours post-dose (prior to the evening dose administration).

On Day 13 a total of 12 blood samples will be drawn for quantification of nalbuphine and its metabolites in plasma from each subject for PK analyses as follows:

- At Day 13 – pre- dose and at 1.50, 3.00, 5.00, 7.00, 9.00, 12.0, 24.0, 36.0, 48.0, 72.0 and 96.0 hours post- last dose.

Trough blood samples will be collected on Days 2, 3, 5, 6, 8, 9, 11, 12, and 13 prior to the morning and evening (if applicable) dose administrations.

Plasma concentrations on Day 1 from nalbuphine and its metabolites will be used to calculate the following parameters by standard non-compartmental methods:

AUC_{0-12}:	Area under the concentration-time curve from time zero to 12 hours.
AUC_{0-24}:	Area under the concentration-time curve from time zero to 24 hours.
C_{max}:	Maximum observed concentration.
T_{max}:	Time of observed C_{max}.

Plasma concentrations on Days 4, 7 and 10 from nalbuphine and its metabolites will be used to calculate the following parameters by standard non-compartmental methods:

AUC_{0-12}:	Area under the concentration-time curve from time zero to 12 hours.
C_{max}:	Maximum observed concentration.
T_{max}:	Time of observed C_{max}.

Plasma concentrations on Day 13 will be used to calculate the following parameters by standard non-compartmental methods:

AUC_{0-12}:	Area under the concentration-time curve from time zero to 12 hours.
AUC_{0-24}:	Area under the concentration-time curve from time zero to 24 hours.
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, calculated as $AUC_{0-t}+C_t/K_{el}$, where: C_t = the last measurable concentration.
C_{max}:	Maximum observed concentration.
C_{trough} :	Concentration at the end of one dosing interval (tau = 12 hours)

T_{max}:	Time of observed C_{max}.
Fl (%):	Percentage of fluctuation (100* (C_{max} – C_{tau})/C_{avg} Where C_{tau} is C_{trough} and Cavg is equal to AUC_{tau}/tau or AUC_{0-12}/12 hours in the current study.
Residual area	calculated as 100*(1- AUC_{0-t} / AUC_{0-inf})
$T_{½\ el}$:	Terminal elimination half-life
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. At least 3 concentration points will be used in estimating K_{el}. C_{max} should not be used in this estimation unless there are only two other quantifiable concentrations available following the C_{max}. The time point where log-linear K_{el} calculation begins ($K_{el\ Lower}$), and the actual sampling time of the last quantifiable concentration used to estimate the K_{el} ($K_{el\ Upper}$) will be reported with the correlation coefficient from the linear regression to calculate K_{el}.
Cl/F:	Apparent total body clearance, calculated as Dose/AUC_{0-inf} for nalbuphine.
V_d/F:	Apparent volume of distribution during terminal phase, calculated as Dose/(K_{el} x AUC_{0-inf}) for nalbuphine.

Additional PK analysis may be performed. Upon the Sponsor's request, PK repeats might be performed. If re-assays are requested for PK reasons, final results will include re-assay values, while results with original values will be presented in an appendix of the report as supportive data.

8.5 Statistical Analyses

Individual and mean (±SD) plasma concentration versus time curves will be presented for both linear and semi-log scales. Descriptive statistics (arithmetic and geometric means, standard deviation [SD], coefficient of variation [CV%], minimum [Min], maximum [Max], and median) of the plasma concentrations versus time will be presented as well for the PK parameters according to hepatic impairment groups included in the study (Mild, Moderate, Severe hepatic impairment, and Control). A graphical description of the relationship between hepatic impairment measures and PK will be presented.

For Part 1 plasma PK parameters, for each dose level separately using the SAS® GLM procedure, ANOVA will be performed on untransformed K_{el} and $T_{½\ el}$ and on ln-transformed AUC_{0-t}, AUC_{0-inf}, and C_{max}, at the alpha level of 0.05 to compare groups (Control, Mild, Moderate, and Severe). Factors incorporated in the model will include Group as a fixed effect. For the analyses of each dose level, the data (dose-normalized for AUCs and C_{max}) for the healthy control subjects group will be included for comparison purpose. The ratios with 90% CIs (Mild/Control, Moderate/Control, and Severe/Control) will be calculated for $AUC_{0-t,}$ AUC_{0-inf}, and C_{max}. Inter-subject Coefficient of variation will be estimated. For these analytes, T_{max} will be

analyzed non-parametrically (using the Hodges-Lehmann method) with point estimates and 90% CIs for the median differences of T_{max} between groups (Mild-Control, Moderate-Control, and Severe-Control).

For Part 2 plasma PK parameters, for each day (as appropriate), using GLM procedures in SAS, ANOVA will be performed on untransformed K_{el} and $T_{½\ el}$ and on ln-transformed $AUC_{0\text{-}t}$, C_{max}, $AUC_{0\text{-}12}$, $AUC_{0\text{-}24}$, $AUC_{0\text{-}inf}$, and C_{trough} at the alpha level of 0.05 for nalbuphine and metabolites. Factors incorporated in the model will include Group as a fixed effect..

Sample code for the procedure in SAS® codes for ANOVA is specified below:

```
proc glm data=basepk;
   class GROUP;
   model VAR = GROUP / ss3 clparm alpha=0.1;
   means GROUP;
   lsmeans GROUP;
  estimate 'Mild vs. Control' GROUP 1 0 0 -1;
  estimate 'Moderate vs. Control' GROUP 0 1 0 -1;
  estimate 'Severe vs. Control' GROUP 0 0 1 -1;
run;
```

Derived calculations obtained from the ANOVA analyses will be performed as per the following:

- Inter Subject CV = 100 * SQRT ($e^{[MSE]}$ - 1);
- Ratio = 100 * $e^{DIFFERENCE}$, where DIFFERENCE is the point estimate of hepatic impairment groups difference (e.g. Mild – Control, Mild – Control, and Severe - Control) on the ln-transformed scale; and
- 90% Confidence Limits = 100 * $e^{(DIFFERENCE \pm t_{(dfResidual)} * SE_{DIFFERENCE})}$.

Sample code for the procedure in SAS® for nonparametric comparison (Mild vs Control):

```
proc npar1way hl alpha=.10 data= basepk;
    where GROUP in ('1','4');
    class GROUP;
    var Tmax;
    exact hl;
    ods select WilcoxonScores HodgesLehmann;
  run;
```

Within each group, dose proportionality will be assessed by a visual assessment of the individual and mean nalbuphine PK parameters.

In the event that hepatic impairment has a clinically relevant effect on nalbuphine PK, the relationship between hepatic impairment and appropriate PK parameters for nalbuphine (e.g., CL/F, AUC_{0-12}, AUC_{0-24}, AUC_{0-t}, AUC_{0-inf}, AUC_{0-t}, C_{trough}, and C_{max}) will be determined by a linear or non-linear regression.

Study results including the graphical description and the relationships between hepatic impairment and relevant pharmacokinetic parameters will be used to elaborate specific dosing recommendations if deemed clinically relevant.

Additional and exploratory statistical analysis may be performed.

8.6 Steady-State Attainment

For Part 2 of the study, a repeated measures analysis of variance (RMA) will be carried out on ln-transformed pre-morning and evening dose (when applicable) concentrations (Days 2, 3, 5, 6, 8, 9, 11, 12, and 13) to determine attainment of steady state.

Repeated measures analysis of the last three pre-dose concentrations (Ln transformed) will be statistically analyzed using General Linear Model (PROC GLM) of SAS® version 9.4 software.

8.7 Pharmacodynamic Analyses

For Part 2 only, the WI-NRS measure will be used to determine the severity of itch experienced by subjects with hepatic impairment at screening. Subjects will complete the two WI-NRS forms (the "Nighttime Itch" and the "Daytime Itch") at the same time during the screening visit and the average will be taken to determine the baseline severity.

For all Cohort 6 subjects, WI-NRS measure will also be performed on a daily basis from Day -1 to Day 16, twice a day, once within an hour of completing their morning and evening meals (if applicable). Subjects will fill in the "Nighttime Itch" form in the morning and the "Daytime Itch" form in the evening.

PD parameters will be summarized at each protocol scheduled time point, by actual treatment. Actual values and actual changes from baseline will be presented.

8.8 Dose Proportionality

8.8.1 Part 1 (Single Ascending Dose)

For nalbuphine, PK parameters AUC_{0-t}, AUC_{0-inf} and C_{max} dose normalized to 0.06 mg will be provided with descriptive statistics for each group. Mean AUC_{0-t}, AUC_{0-inf} and C_{max} versus Dose will be presented graphically.

8.8.2 Part 2 (Multiple Ascending Dose)

For nalbuphine, PK parameters AUC_{0-t}, C_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-inf}, and C_{trough} dose normalized to 0.06 mg will be provided with descriptive statistics for each group. Mean AUC_{0-t}, C_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-inf}, and C_{trough} versus Dose will be presented graphically.

A power model analysis may also be performed to evaluate the doses-exposure, if warranted.

The power model will be used to investigate the dose proportionality using appropriate data from each Dose. For a PK parameter (P), the power model for a parallel design is

$$P = a \times Dose^{b}$$

where *a* is a multiplicative coefficient of the power model (it is related to the intercept when the model is ln-transformed) and b is the exponential coefficient of the power model (it corresponds to the slope when the model is ln-transformed). The 90% confidence intervals (CI) for b will be computed. The calculations will be performed on the ln-transformed scale using the Mixed procedure in SAS® for AUC_{0-t}, C_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-inf}, and C_{trough}.

Dose proportionality will be evaluated prospectively according to the recommendations found in Hummel et al. (2009). For AUC_{0-t}, C_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-inf}, and C_{trough} the 90% confidence intervals of b will be compared to the following exploratory bounds that depend on the dose range ratio (r=highest dose / lowest dose):

Lower bound = $1+\ln(0.5)/\ln(r)$ and

Upper bound = $1+\ln(2)/\ln(r)$.

However, a more stringent criterion will be needed to conclude definitely at dose proportionality. Mean AUC_{0-t}, C_{max}, AUC_{0-12}, AUC_{0-24}, AUC_{0-inf}, and C_{trough} versus Dose will be presented graphically.

9. 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 correlation (Corr.) data: rounded off to four decimal digits.
- Pharmacokinetic parameters related to time such as T_{max}, $K_{el\ Lower}$, and $K_{el\ Upper}$ 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.
- Ratios and 90% confidence intervals, and inter-CV (%) will be presented to two decimal places.

10. Data Handling

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

11. Handling of Missing Data

For PK, only observed data will be used in the data analysis except for concentration values BLQ and samples with no reportable value occurring prior to dosing as described in Section 8.2. No attempt will be made to extrapolate or interpolate estimates for 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 as a TEAE.
- If an AE is recorded with an onset date that does not correspond to the dosing day, but the time is missing, then the AE will be assigned to the treatment as a TEAE if AE onset date is after dosing date.
- If an AE is recorded with an onset date where day and time are both missing, then the AE allocation to the treatment will be done on a case by case basis considering available information (e.g. AE end date, AE comments, subject disposition).

12. Software to be Used

PK analysis will be performed using Phoenix WinNonlin® version 8.0 or higher, which is validated by Syneos. 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). The report text will be created using Microsoft® Office Word 2010, or a higher version.

13. Reference List

- Chuang-Stein C. Summarizing laboratory data with different reference ranges in multi-center clinical trials. Drug Information Journal. 1992; 26:77-84.
- Karvanen J. The statistical basis of laboratory data normalization. Drug Information Journal. 2003; 37:101-107.
- Hummel J., McKendrick S., Brindley C. and French, R. Exploratory assessment of dose proportionality: review of current approaches and proposal for a practical criterion. Pharmaceut. Statist. 2009; 8: 38-49.