Protocol

AM1476 - A Phase I, Double-blind, Placebo-controlled, Single- and Multiple-oral Dose, Safety, Tolerability, and Pharmacokinetic Study in Healthy Subjects

Protocol Status: Final
Protocol Date: 06 January 2021
Protocol Version: 5

Investigational Medicinal Product: AM1476

Protocol Reference Number: 1476-291-001
Covance Study Number: 8417263
EudraCT Number: 2019-004800-37

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SPONSOR APPROVAL

I have read the protocol and approve it:

INVESTIGATOR AGREEMENT

I have read the protocol and agree to conduct the study as described herein

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SYNOPSIS

Study Title

AM1476 - A Phase I, Double-blind, Placebo-controlled, Single- and Multiple-oral Dose, Safety, Tolerability, and Pharmacokinetic Study in Healthy Subjects

Objectives

The primary objective of the study is:

- to determine the safety and tolerability of single- and multiple-oral doses of AM1476 in healthy subjects.

The secondary objectives of the study are:

- to determine the single- and multiple-oral dose pharmacokinetics (PK) of AM1476 in healthy subjects.
- to determine the effect of food on the single oral dose PK of AM1476 in healthy subjects.

Study Design

This will be a double-blind, randomised, placebo-controlled, single- and multiple-oral dose study conducted in 2 parts.

Part A will comprise a single-ascending dose, sequential-group design incorporating a single-group, 2-period crossover arm incorporating a food-effect evaluation.

Overall, 48 subjects will be studied in 6 groups (Groups A1 to A6), with each group consisting of 8 subjects.

Each subject will participate in 1 treatment period only, except for Group A3, where each subject will participate in 2 treatment periods separated by a minimum of 6 days. Subjects will reside at the Clinical Research Unit (CRU) from Day -1 (the day before dosing) to Day 3 of each treatment period, as applicable. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

In each of Groups A1 to A6, 6 subjects will receive AM1476 and 2 subjects will receive placebo. In Groups A1, A2, and A4 to A6, all doses will be administered in the fasted state in accordance with a randomisation schedule on the morning of Day 1. For Group A3, Treatment Period 1, Day 1 doses will be administered in the fasted state in accordance with a randomisation schedule and Treatment Period 2, Day 1 doses will be given 30 minutes after starting a standard high-fat breakfast. Each subject in Groups A1, A2, and A4 to A6 will receive only a single dose of AM1476 or placebo during the study. In Group A3, subjects will have the same treatment in both periods, such that each subject will receive 2 single doses of AM1476 or placebo during the study. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.

All groups in Part A will be divided into 2 cohorts, with each cohort being dosed at least 24 hours apart. The first cohort will comprise 2 subjects, with 1 subject receiving AM1476 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving AM1476 and 1 subject receiving placebo. For groups participating in a food-effect evaluation, sentinel dosing will only be utilised in Treatment Period 1 when AM1476 or placebo are administered in the fasted state. Dosing of subjects in the second cohort will not continue if any of the dose escalation stopping criteria are met by the sentinel subjects (first cohort).

There will be a minimum of 6 days between dose escalations for each group.

Part B will comprise a multiple-ascending dose, sequential-group design. Overall, 24 subjects will be studied in 3 groups (Groups B1 to B3), with each group consisting of 8 subjects. Part B may start following review of the safety and tolerability and PK data for Group A4, at a dose equal or less than given in Groups A1 to A4, but cannot be equal to a dose level from Part A if that dose met any of the dose escalation stopping rules.

Each subject will participate in 1 treatment period only and reside at the CRU from Day -1 until the morning of Day 12. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

In each of Groups B1 to B3, 6 subjects will receive AM1476 and 2 subjects will receive placebo. The dietary state for dosing in Part B will be subject to review of the PK data from the fed/fasted comparison in Part A. For all subjects, dosing is planned to be once daily (QD) on Days 1 to 10, inclusive. However, the dietary state (including fasting requirement and meal compositions), dosing duration, and dosing frequency may be changed following review of safety, tolerability, and PK data from Part A or earlier groups in Part B. The dose regimen will comprise no less than once every 2 days and will not exceed twice daily (BID) dosing. The total daily dose administered will not exceed an exposure shown to be safe and well-tolerated in Part A. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days. There will be a minimum of 6 days between dose escalations for each group.

Number of Subjects

Part A: 48 subjects will be studied in 6 groups (Groups A1 to A6).

Part B: 24 subjects will be studied in 3 groups (Groups B1 to B3).

Additional groups: following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria) may be added to the study. There will be no further dose escalation in these additional groups if dose escalation stopping criteria have been met and a dose level cannot be repeated if it previously met a stopping criterion. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in each of Parts A and B.

Diagnosis and Main Criteria for Inclusion

Healthy male and female subjects aged between 18 and 60 years (inclusive) with a body mass index (BMI) between 18.0 and 32.0 kg/m^2 (inclusive).

Investigational Medicinal Products, Dose, and Mode of Administration

Proposed dose levels for Part A: 1 mg for Group A1; subsequent dose levels are to be determined following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A and Day 10 in Part B]) from the lower dose levels.

Proposed dose levels for Part B: the dose levels, dosing frequency, and dosing duration for Part B will be decided, in consultation with the Sponsor, on the basis of data from Part A of the study or earlier groups in Part B. The total daily exposure of AM1476 administered during this part of the study will be planned to not exceed an exposure shown to be safe and well-tolerated in Part A.

Administration route: oral.

Reference Product and Mode of Administration

Reference product: placebo capsules.

Administration route: oral.

Duration of Subject Participation in the Study

Part A
Planned Screening duration: approximately 4 weeks (approximately 5 weeks for Group A1).

Planned study duration (Screening to Follow-up): approximately 6 weeks for subjects not participating in the food-effect evaluation (approximately 7 weeks for Group A1) and approximately 7 weeks for those participating in the food-effect evaluation.

Part B
Planned Screening duration: approximately 4 weeks.

Planned study duration (Screening to Follow-up): approximately 7 weeks.

Endpoints

Safety:

Adverse events (AEs), clinical laboratory evaluations (clinical chemistry, haematology, coagulation, and urinalysis), vital signs measurements, 12-lead electrocardiograms (ECGs), telemetry, neurological examinations, Columbia-Suicide Severity Rating Scale (C-SSRS), and physical examinations.

Pharmacokinetics:

Blood and urine samples for the analysis of plasma and urinary concentrations of AM1476. Pharmacokinetic parameters will be derived by non-compartmental analysis.

In Part A, the PK parameters will include area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$), area under the plasma concentration-time curve from

time zero to the time of the last quantifiable concentration ($AUC_{0\text{-}tlast}$), maximum observed plasma concentration (C_{max}), time of the maximum observed plasma concentration (t_{max}), apparent plasma terminal elimination half-life ($t_{½}$), apparent total plasma clearance (CL/F), apparent volume of distribution (V_z/F), renal clearance (CL_R), amount of drug excreted in the urine (A_e), and percentage of dose excreted unchanged in urine (F_e).

In Part B, the PK parameters will include: area under the plasma concentration-time curve over a dosing interval ($AUC_{0\text{-}\tau}$), $AUC_{0\text{-}\infty}$ (Day 1 only), C_{max}, minimum observed plasma concentration (C_{min}), t_{max}, $t_{½}$, CL/F, V_z/F, observed accumulation ratio based on $AUC_{0\text{-}\tau}$ ($RA_{AUC0\text{-}\tau}$), and observed accumulation ratio based on C_{max} (RA_{Cmax}), CL_R, A_e, and F_e.

Other PK parameters may also be reported.

Statistical Methods

Safety:

Safety parameters will be listed and summarised using descriptive statistics. No formal statistical analysis of safety data is planned.

Pharmacokinetics:

Individual plasma and urine concentrations of AM1476 and plasma and urine PK parameters will be listed and summarised using descriptive statistics. Individual and mean AM1476 concentration-time profiles will be presented graphically.

In Part A, where data are available, AM1476 dose proportionality will be examined across the dose groups. The PK parameters will be analysed for dose proportionality using a power model approach or analysis of variance (ANOVA) model as appropriate. Where data are available, the effect of food at 1 dose level in Part A will be investigated using ANOVA.

In Part B, $AUC_{0\text{-}\tau}$ and C_{max} on Day 10 will be analysed for dose proportionality using a power model approach or ANOVA model as appropriate.

To investigate the effect of food on the PK of AM1476, the log-transformed $AUC_{0\text{-}\infty}$, $AUC_{0\text{-}tlast}$, and C_{max} values for AM1476 administered with and without food will be analysed using a linear mixed-effects model with treatment as fixed effect and subject as a random effect. Point estimates and 90% confidence intervals (CIs) for differences between the treatments (fed versus fasted) on the log scale will be exponentiated to obtain estimates for the ratios of geometric means and respective 90% CIs on the original scale.

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LIST OF TABLES AND FIGURES

LIST OF ABBREVIATIONS

Abbreviation	Definition
5-HT	5-hydroxytryptamine
5-HT_{2B}	5-hydroxytryptamine receptor 2B
A_e	amount of drug excreted in the urine
AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANOVA	analysis of variance
API	active pharmaceutical ingredient
AST	aspartate aminotransferase
AUC_{0-24}	area under the plasma concentration-time curve from time zero to 24 hours postdose
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{0-tlast}$	area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration
$AUC_{0-\tau}$	area under the plasma concentration-time curve over a dosing interval
BID	twice daily
BMI	body mass index
bpm	beats per minute
CFR	Code of Federal Regulations
cGvHD	chronic graft versus host disease
CI	confidence interval
CIOMS	Council for International Organizations of Medical Sciences
CL/F	apparent total plasma clearance
CL_R	renal clearance
C_{max}	maximum observed plasma concentration
C_{min}	minimum observed plasma concentration
CRO	Contract Research Organisation
CRU	Clinical Research Unit
C-SSRS	Columbia-Suicide Severity Rating Scale
[redacted]	[redacted]
DSS	Drug Safety Services
EC	Ethics Committee
ECG	electrocardiography/electrocardiogram
eCRF	electronic Case Report Form
EDC	electronic data capture
F_e	percentage of drug excreted in the urine
FSH	follicle-stimulating hormone

GCP	Good Clinical Practice
[redacted]	[redacted]
GGT	gamma-glutamyl transferase
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HED	human equivalent dose
hERG	human-ether-à-go-go-related gene
HIV	human immunodeficiency virus
IB	Investigator's Brochure
IC_{50}	half maximal inhibitory concentration
ICF	Informed Consent Form
ICH	International Council for/Conference on Harmonisation
IMP	investigational medicinal product
IUD	intrauterine device
[redacted]	[redacted]
N	number of subjects
NA	not applicable
NC	not calculated
NOAEL	no-observed-adverse-effect level
[redacted]	[redacted]
NR	not reported
PBPK	physiologically based pharmacokinetic
[redacted]	[redacted]
PK	pharmacokinetic(s)
[redacted]	[redacted]
pSmad3	phosphorylated Smad3
QD	once daily
QTc	QT interval corrected for heart rate
QTcF	QT interval corrected for heart rate using Fridericia's method
$RA_{AUC0-\tau}$	observed accumulation ratio based on $AUC_{0-\tau}$
RA_{Cmax}	observed accumulation ratio based on C_{max}
RBC	red blood cell
SAE	serious adverse event
SAP	Statistical Analysis Plan
SMF	Site Master File
SSc	systemic sclerosis
$t_{½}$	apparent plasma terminal elimination half-life
TBD	to be determined
TGF	transforming growth factor
t_{max}	time of the maximum observed plasma concentration

TMF	Trial Master File
TSE	Transmissible Spongiform Encephalopathy
Tsk-1	tight-skin-1
ULN	upper limit of normal
V_z/F	apparent volume of distribution
WBC	white blood cell

1. INTRODUCTION

1.1. Overview

AM1476 is a 5-hydroxytryptamine receptor 2B (5-HT_{2B}) antagonist being developed by AnaMar AB for the treatment of systemic sclerosis. Peripheral 5-HT_{2B} receptors have been suggested to play a significant role in fibrosis, with the receptor being upregulated in fibrotic tissues. Activation of the 5-HT_{2B} receptor leads to increased production of profibrotic mediators, modifying cell differentiation leading to excessive extracellular matrix synthesis and eventually fibrosis.[1–3]

Due to the high number of affected individuals, incomplete knowledge of the fibrotic process pathogenesis, the marked heterogeneity in their aetiology and clinical manifestations, the absence of appropriate and fully validated biomarkers, and most importantly, the current lack of effective disease-modifying therapeutic agents, human fibrotic diseases represent a major global health problem. Despite many differences, the fibrotic diseases share the common feature of an uncontrolled and progressive accumulation of fibrotic tissue in affected organs and numerous studies have identified activated myofibroblasts as the cells ultimately responsible for the replacement of normal tissues with non-functional fibrotic tissue. Critical signalling cascades, initiated primarily by transforming growth factor (TGF)-β, but also involving numerous cytokines and signalling molecules, such as 5-hydroxytryptamine (5-HT), which stimulate pro-fibrotic reactions in myofibroblasts, offer potential therapeutic targets.[4]

Serotonin (5-HT) is well-established scientifically as a stimulator of tissue fibrosis.[5]

The 5-HT_{2B} receptor, therefore, represents a promising target for new anti-fibrotic treatments. The development of new drugs targeting the 5-HT_{2B} receptor has so far been hampered by non-selective compounds with unwanted side effects. New, safe, and selective 5-HT_{2B} receptor antagonists are therefore needed.

1.2. Summary of Non-clinical Pharmacology

AM1476 has been evaluated for 5-HT_{2B} receptor interactions using mouse, rat, and human receptors. Both binding (human only) assays and functionality assays have been employed.

In a chronic graft versus host disease (cGvHD) model of systemic sclerosis (SSc) using minor histocompatibility antigen-mismatched syngeneically and allogeneically transplanted mice, statistically significant effects on readouts of fibrosis (dermal and pulmonary fibrosis and reduced number of cells positive for phosphorylated Smad3 [pSmad3]) were observed following oral administration of AM1476 at 1 mg/kg, 10 mg/kg, or 30 mg/kg twice daily (BID) or 10 mg/kg once daily (QD). Inflammation-driven manifestations such as weight loss or the cutaneous skin score were not significantly affected by treatment. In the tight-skin-1 (Tsk-1) mouse model, oral administration of 1 mg/kg or 10 mg/kg BID AM1476 or 10 mg QD resulted in statistically significant and highly potent amelioration of skin fibrosis and a reduced number of cells positive for pSmad3.

1.3. Summary of Safety Pharmacology

Five safety pharmacology studies (4 Good Laboratory Practice [GLP] and 1 non-GLP) were conducted. AM1476 had no noteworthy effects on behaviour, body temperature, or respiration in mice, and the no-observed-effect level (NOEL) in these studies was considered to be 180 mg/kg (oral administration).

The half maximal inhibitory concentration (IC_{50}) in a human-ether-à-go-go-related gene (hERG) assay was 30.12 µM (8.21 µg/mL). When tested versus 8 ion channels, AM1476 did not inhibit any ion channel currents by greater than 20% normalised inhibition at 10 µM.

[redacted]

1.4. Summary of Genotoxicology

The genotoxic and mutagenic potential of AM1476 was investigated in standard GLP in vitro (bacterial mutagenicity and human lymphocyte micronucleus) and in vivo (mouse bone marrow micronucleus assay) studies.

[redacted]

1.5. Summary of Development and Reproductive Toxicity

Embryo-foetal development studies have been conducted in the mouse and rabbit.

[redacted]

[redacted]

[redacted]

1.6. Summary of Toxicology

Pivotal repeat-dose toxicology studies were conducted in CD-1 mice and beagle dogs given oral AM1476 for 28 days followed by a 4-week recovery period and for up to 13 weeks. No single-dose toxicity studies have been conducted.

1.6.1. 28-day Oral Toxicity Study with a 4- or 6-week Recovery Period in Mice (GLP Study)

This pivotal toxicology study in mice was conducted in accordance with GLP, in which AM1476 was administered orally to Crl:CD1(ICR) mice for 28 days to determine toxicity. Mice were administered 0 mg/kg/day, 90 mg/kg/day, 180 mg/kg/day, or 360 mg/kg/day. The 4-week (Group 5) and 6-week (Group 1) recovery phase enabled an assessment of reversibility, or persistence, of any effects. The toxicokinetic profile of the test article was also assessed.

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1.6.2. 13-Week Oral Toxicity Study in Mice (GLP Study)

This study in mice was conducted in accordance with GLP, in which AM1476 was administered orally to Crl:CD1(ICR) mice (12/sex/toxicity groups; 3 [control] or 18/sex/toxicokinetic groups) for 13 weeks to determine toxicity and toxicokinetic profile. Mice were administered 0 mg/kg/day, 45 mg/kg/day, 90 mg/kg/day, or 180 mg/kg/day.

[redacted]

1.6.3. 28-day Oral Toxicity Study with a 4-week Recovery Period in Dogs (GLP Study)

This pivotal toxicology study in dogs was conducted in accordance with GLP, in which AM1476 was administered orally at 0 mg/kg/day (vehicle control), 15 mg/kg/day, 30 mg/kg/day, or 65 mg/kg/day to beagle dogs (5/sex in the control and high-dose groups, 3/sex in the low- and mid-dose groups) for 28 days to evaluate its toxicity, and to assess the reversibility, persistence, or delayed occurrence of any effects after a 4-week recovery phase. The toxicokinetic profile of the test article was also assessed.

[redacted]

1.6.4. 13-Week Oral Toxicity Study in Dogs (GLP Study)

This study in dogs was conducted in accordance with GLP, in which AM1476 was administered orally to beagle dogs (3/sex/groups) for 13 weeks to determine toxicity and

toxicokinetic profile. Dogs were administered 0 mg/kg/day, 15 mg/kg/day, 30 mg/kg/day, or 65/50 mg/kg/day [redacted]

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1.7. Summary of Non-clinical Pharmacokinetics

Generally, sex differences in C_{max} and AUC_{0-24} values were less than 2-fold. Exposure, as assessed by C_{max} and AUC_{0-24}, generally increased with the increasing dose level. The increases in C_{max} and AUC_{0-24} were approximately dose-proportional at lower dose levels and less-than-dose-proportional at higher dose levels. Overall, there was no accumulation of AM1476 observed after multiple doses in both mice and dogs, except after multiple administrations of 65 mg/kg/day in the dog.

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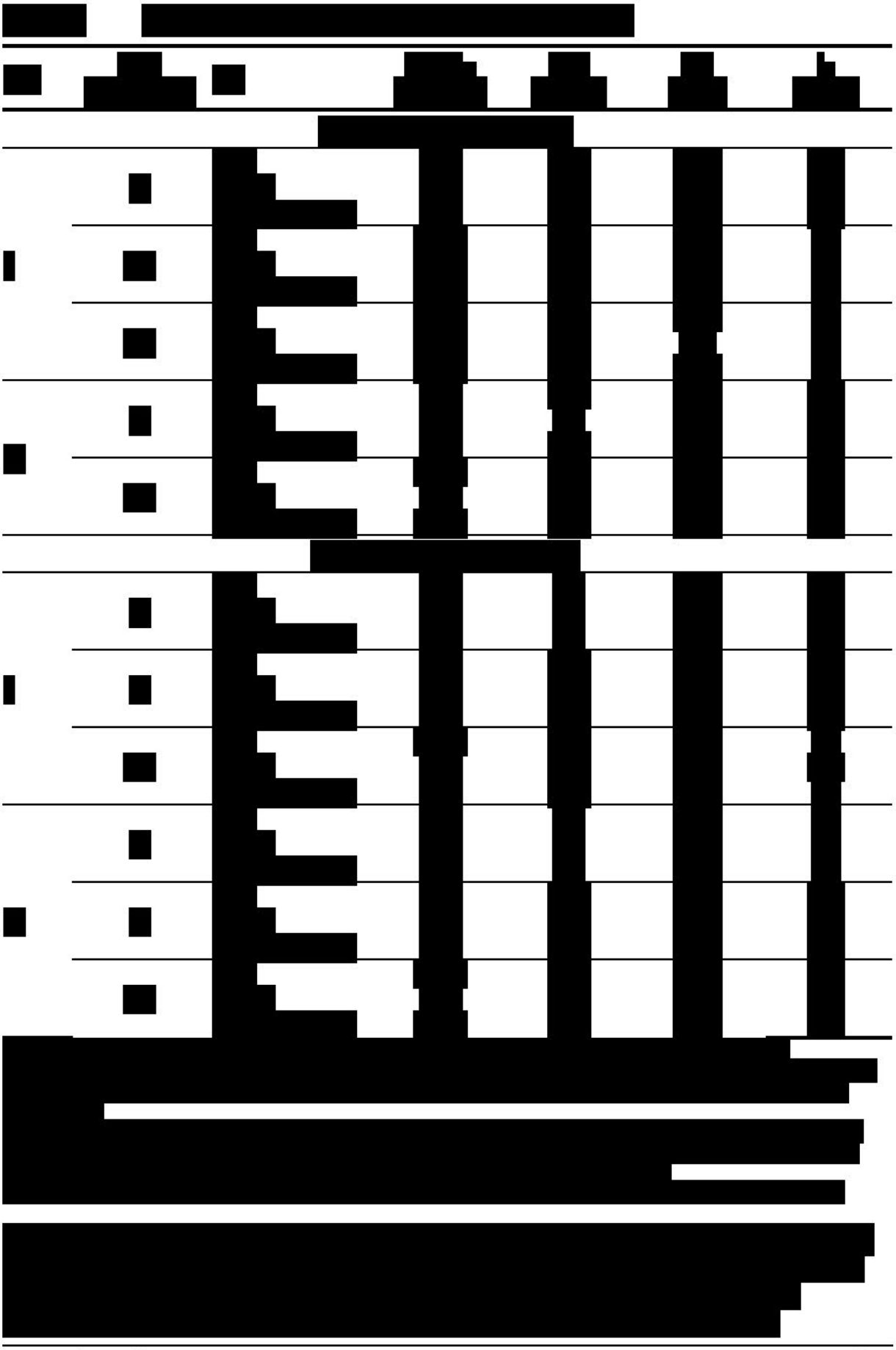
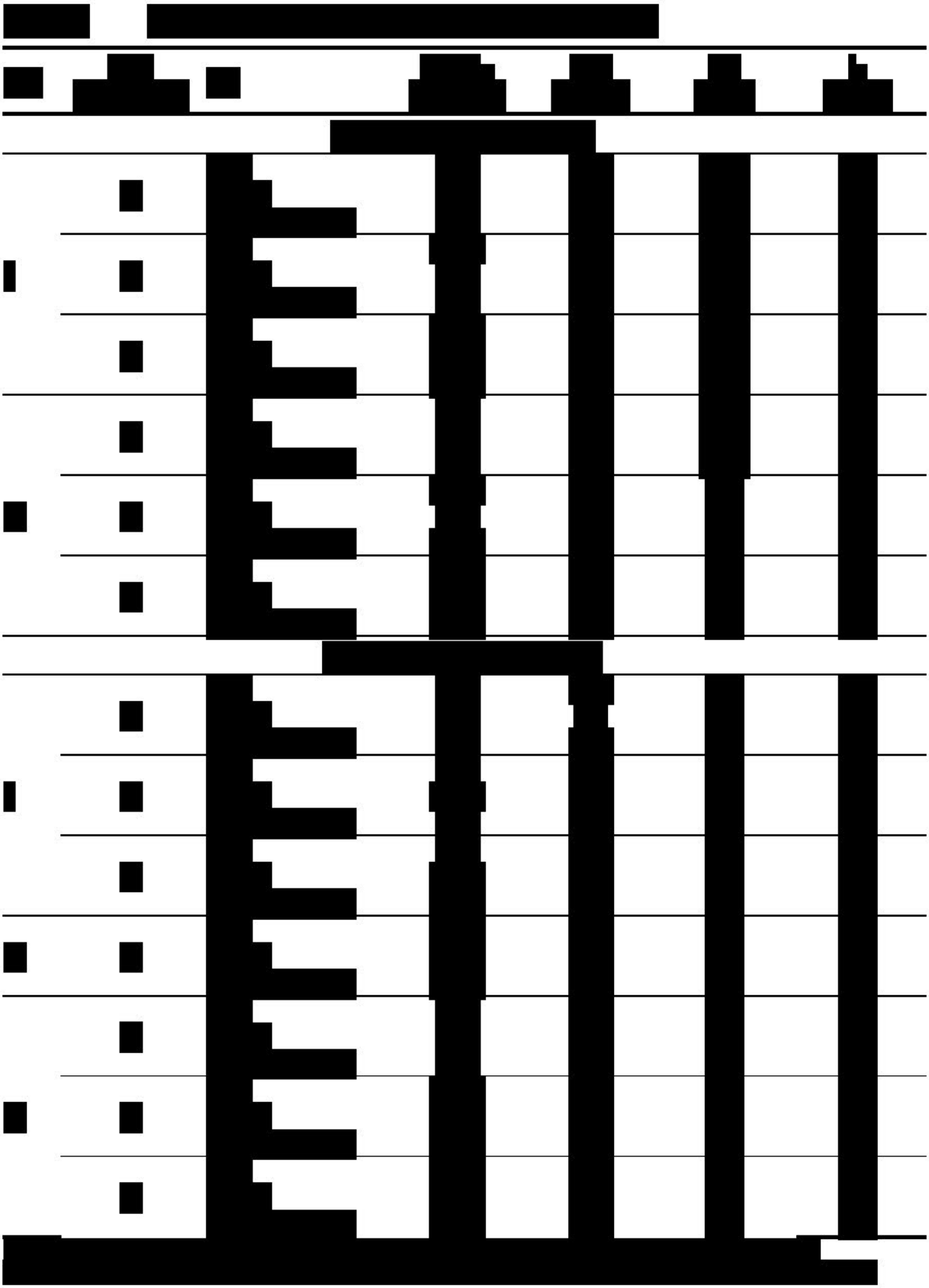




1.8. Summary of Clinical Experience

No clinical studies have been conducted with AM1476.

1.9. Study Rationale

This is the first time AM1476 will be administered to humans. The principal aim of this study is to obtain safety and tolerability data when AM1476 is administered orally as single and multiple doses to healthy subjects. This information, together with the pharmacokinetic (PK) data, will help establish the doses and dosing regimen suitable for future studies in patients. The study will also investigate the effect of food on the PK of AM1476 prior to patient studies.

1.10. Benefit-risk Assessment

Healthy subjects in the current study will not receive any health benefit (beyond that of an assessment of their medical status) from participating in the study. The risks of participation are primarily those associated with adverse reactions to the investigational medicinal product (IMP), although there may also be some discomfort from collection of blood samples and other study procedures. More information about the known and expected benefits, risks, and reasonably anticipated adverse events (AEs) associated with AM1476 may be found in the Investigator's Brochure (IB).[6]

2. OBJECTIVES AND ENDPOINTS

2.1. Objectives

The primary objective of the study is:

- to determine the safety and tolerability of single and multiple oral doses of AM1476 in healthy subjects.

The secondary objectives of the study are:

- to determine the single- and multiple-oral dose PK of AM1476 in healthy subjects.
- to determine the effect of food on the single-oral dose PK of AM1476 in healthy subjects.

2.2. Endpoints

2.2.1. Primary Endpoints

The primary safety endpoints for this study are as follows:

- incidence and severity of AEs
- incidence of laboratory abnormalities, based on haematology, clinical chemistry, coagulation, and urinalysis test results
- vital signs measurements
- 12-lead ECG parameters
- telemetry
- neurological examinations
- Columbia-Suicide Severity Rating Scale (C-SSRS)
- physical examinations.

2.2.2. Secondary Endpoints

For Part A, the single-ascending dose and food (fed versus fasted dietary status at dosing), PK outcome endpoints of AM1476 are as follows:

- area under the plasma concentration-time curve from time zero to infinity ($AUC_{0-\infty}$)
- area under the plasma concentration-time curve from time zero to the time of the last quantifiable concentration ($AUC_{0-tlast}$)
- maximum observed plasma concentration (C_{max})
- time of the maximum observed plasma concentration (t_{max})
- apparent plasma terminal elimination half-life ($t_{½}$)

- apparent total plasma clearance (CL/F)
- apparent volume of distribution (V_z/F)
- renal clearance (CL_R)
- amount of drug excreted in the urine (A_e)
- percentage of drug excreted in the urine (F_e).

For Part B, the multiple-ascending dose PK outcome endpoints of AM1476 are as follows:

- area under the plasma concentration-time curve over a dosing interval ($AUC_{0-\tau}$)
- $AUC_{0-\infty}$ (Day 1 only)
- C_{max}
- minimum observed plasma concentration (C_{min})
- t_{max}
- $t_{½}$
- CL/F
- V_z/F
- observed accumulation ratio based on $AUC_{0-\tau}$ ($RA_{AUC0-\tau}$)
- observed accumulation ratio based on C_{max} (RA_{Cmax})
- CL_R
- A_e
- F_e.

Other PK parameters may also be reported.

3. INVESTIGATIONAL PLAN

3.1. Overall Study Design and Plan

This will be a double-blind, randomised, placebo-controlled, single- and multiple-oral dose study conducted in 2 parts.

3.1.1. Part A

Part A will comprise a single-ascending dose, sequential-group design incorporating a single-group, 2-period crossover arm incorporating a food-effect evaluation. Overall, 48 subjects will be studied in 6 groups (Groups A1 to A6), with each group consisting of 8 subjects.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration (within 35 days for Group A1). Each subject will participate in 1 treatment period only, except for those participating in a food-effect

evaluation, where each subject will participate in 2 treatment periods separated by a minimum of 6 days.

Subjects will reside at the Clinical Research Unit (CRU) from Day -1 (the day before dosing) to Day 3 of each treatment period, as applicable. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

Based on the ongoing review of the safety, tolerability, and PK results, additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 per period and will not extend beyond 28 days after each final dosing occasion.

In each of Groups A1 to A6, 6 subjects will receive AM1476 and 2 subjects will receive placebo.

Groups A1, A2, and A4 to A6
It is planned for each subject in Groups A1, A2, and A4 to A6 to receive only a single dose of AM1476 or placebo during the study. Doses will be administered in the fasted state in accordance with a randomisation schedule on the morning of Day 1.

Group A3
It is planned for each subject in Group A3 to have the same treatment in both treatment period such that each subject will receive 2 single doses of AM1476 or placebo during the study. On Day 1 in Treatment Period 1, doses will be administered in the fasted state in accordance with a randomisation schedule. On Day 1 in Treatment Period 2, doses will be given 30 minutes after starting a standard high-fat breakfast. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.

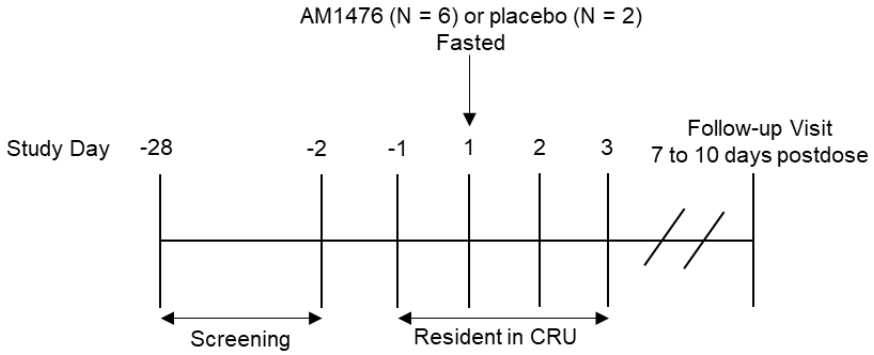
Additional Groups (Groups A7 to A9)
If it is decided to enrol additional groups (Section 3.3), the effect of food on the PK of AM1476 may further be evaluated as described for Group A3. However, fasting requirements, meal compositions, and timing of doses will be determined following review of the available PK data.

Sentinel Dosing
All groups in Part A will be divided into 2 cohorts, with each cohort being dosed at least 24 hours apart. The first cohort will comprise 2 subjects, with 1 subject receiving AM1476 and 1 subject receiving placebo. The second cohort will comprise 6 subjects, with 5 subjects receiving AM1476 and 1 subject receiving placebo. For groups participating in a food-effect evaluation, sentinel dosing will only be utilised in Treatment Period 1 when AM1476 or placebo are administered in the fasted state. Dosing of subjects in the second cohort will not continue if any of the dose escalation stopping criteria (Section 3.7) are met by the sentinel subjects (first cohort).

The total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 6 weeks for subjects not participating in the food-effect evaluation (approximately 7 weeks for Group A1) and approximately 7 weeks for those participating in the food-effect evaluation.

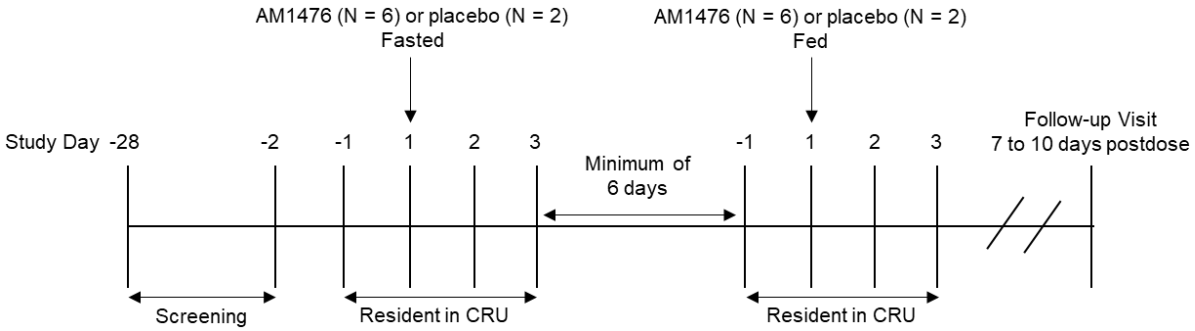
An overview of the study design is shown in Figure 1 and Figure 2, and the planned dose levels in Figure 3. A Schedule of Assessments is presented in Appendix 6.

Figure 1: Study Schematic (Part A) for Groups A1, A2, and A4 to A6



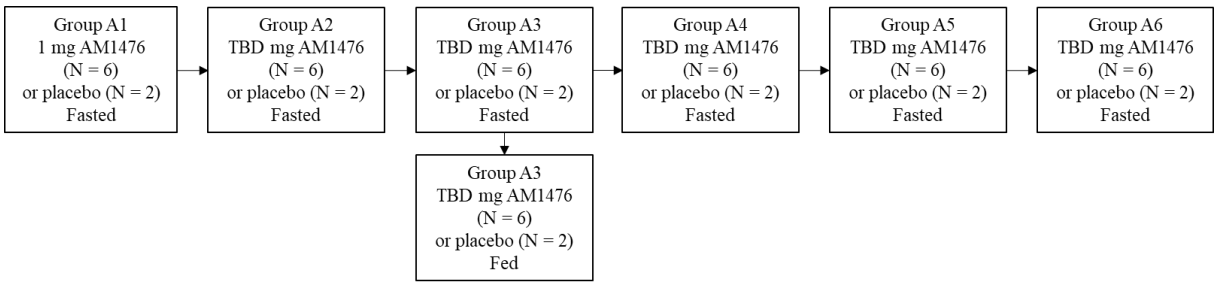
Abbreviations: CRU = Clinical Research Unit; N = number of subjects.
Note: Group A1: Screening period from Day -35 to Day -2 (approximately 5 weeks)

Figure 2: Study Schematic (Part A) for Food-effect Evaluation(s)



Abbreviations: CRU = Clinical Research Unit; N = number of subjects.

Figure 3: Planned Dose Levels (Part A)



Abbreviations: N = number of subjects; PK = pharmacokinetic(s); TBD = to be determined.
NOTE: dose levels may be adjusted based on the ongoing review of the safety, tolerability, and PK data. Doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A]) from the lower dose levels.
Although the food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.

3.1.2. Part B

Part B will comprise a multiple-ascending dose, sequential-group design. Overall, it is planned for 24 subjects to be studied in 3 groups (Groups B1 to B3), with each group

consisting of 8 subjects. Part B may start following review of the safety and tolerability and PK data for Group A4, at a dose equal or less than given in Groups A1 to A4, provided the predicted exposure is not expected to exceed an exposure already shown to be safe and well tolerated. The dose level cannot be equal to a dose level from Part A if that dose met any of the dose escalation stopping rules.

Potential subjects will be screened to assess their eligibility to enter the study within 28 days prior to the first dose administration. Each subject will participate in 1 treatment period only and reside at the CRU from Day -1 until the morning of Day 12. All subjects will return for a Follow-up visit 7 to 10 days after their final dose.

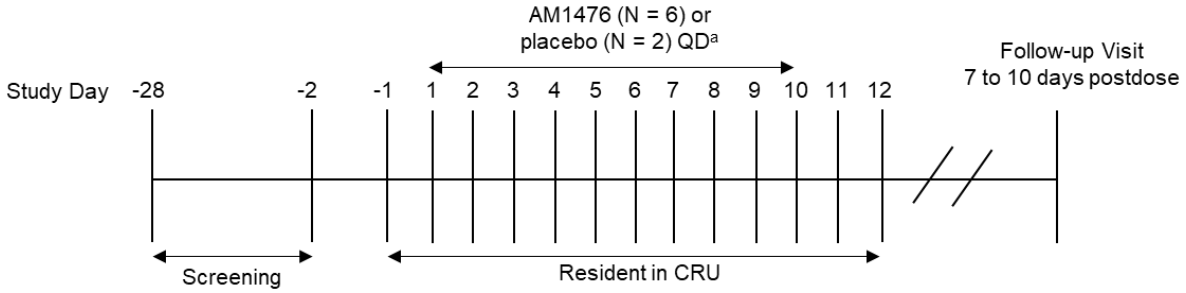
Based on the ongoing review of available safety, tolerability, and PK data, up to 3 additional non-residential visits may be required. The number of additional visits per subject will not exceed 3 and will not extend beyond 28 days after each final dosing occasion.

In each of Groups B1 to B3, 6 subjects will receive AM1476 and 2 subjects will receive placebo. The dietary state for dosing in Part B will be subject to review of the PK data from the fed/fasted comparison in Part A. For all subjects, dosing is planned to be QD on Days 1 to 10, inclusive. However, the dietary state (including fasting requirement and meal compositions), dosing duration, and dosing frequency may be changed following review of safety, tolerability, and PK data from Part A or earlier groups in Part B (Section 3.4). The dose regimen will comprise no less than once every 2 days and will not exceed BID dosing. The predicted total daily exposure at the selected dose administered will not exceed an exposure shown to be safe and well-tolerated in Part A. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days. There will be a minimum of 6 days between dose escalations for each group.

The total duration of study participation for each subject (from Screening through Follow-up visit) is anticipated to be approximately 7 weeks.

An overview of the study design is shown in Figure 4, and the planned dose levels in Figure 5. A Schedule of Assessments is presented in Appendix 7.

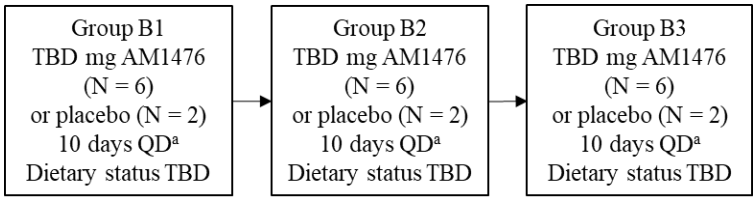
Figure 4: Study Schematic (Part B)



Abbreviations: BID = twice daily; CRU = Clinical Research Unit; N = number of subjects; QD = once daily.

a. Dosing is planned to be QD on Days 1 to 10, inclusive. The dosing regimen in Part B may be changed following review of data from groups in Part A or earlier groups in Part B. The dose regimen will comprise no less than once every 2 days and will not exceed BID dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days.

Figure 5: Planned Dose Levels (Part B)



Abbreviations: BID = twice daily; N = number of subjects; QD = once daily; TBD = to be determined.
NOTE: doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A and Day 10 in Part B]) from Part A or earlier groups in Part B.
a. Dosing is planned to be QD on Days 1 to 10, inclusive. The dosing regimen in Part B may be changed following review of data from groups in Part A or earlier groups in Part B and will not exceed BID dosing.

3.2. Study Start and End of Study Definitions

The start of the study is defined as the date the first subject signs an Informed Consent Form (ICF). The point of enrolment occurs at the time of subject number allocation. The end of the study is defined as the date of the last subject's last assessment (scheduled or unscheduled).

3.3. Additional Groups

Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. There will be no further dose escalation in these additional groups if dose escalation stopping criteria have been met and a dose level cannot be repeated if it previously met a stopping criterion. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in each of Parts A and B. The requirement for additional groups will be agreed with the Sponsor and documented in the Trial Master File (TMF).

3.4. Discussion of Study Design, Including the Choice of Control Groups

For both parts of the study, a sequential-group, ascending-dose design has been chosen for safety reasons as AM1476 is in the early stages of clinical development, with Part A of the study being the first time it will be administered to humans. Oral doses have been chosen for both parts of the study, as this is the intended clinical route of administration. A 2-period crossover design has been chosen for the food-effect arm, as this gives a within-subject assessment of the influence of food on the PK of AM1476 and so increases the power of the study for the given number of subjects.

It is the intent of Part B to dose subjects such that steady-state plasma levels of AM1476 are achieved and maintained for several days. Based on the available non-clinical data, it is expected that this will be achieved following 10 days of QD dosing; however, a full review of all the safety, tolerability, and PK data from Part A will be performed to confirm the dose regimen for Part B. If the $t_{½}$ of AM1476 is shorter than predicted by the non-clinical data, BID dosing over 7 days may be appropriate. If the $t_{½}$ of AM1476 is longer than predicted by the non-clinical data, QD dosing may be more appropriate over 28 days (as steady-state will take longer to achieve). The dose regimen will comprise no less than once every 2 days and will not exceed BID dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days of dosing.

Details of the dosing regimen and duration used for Part B of the study will be documented in the TMF.

Based upon the non-clinical data, the duration of each treatment period is considered adequate to achieve the study objectives. Where applicable, an interval of at least 6 days between treatment periods in the food-effect arm is considered adequate to prevent carryover of AM1476.

This study will be double-blind and placebo-controlled in order to avoid bias in the collection and evaluation of data during its conduct. Placebo has been chosen as the control treatment to assess whether any observed effects are treatment-related or simply reflect the study conditions.

Conducting the study in healthy subjects mitigates the potential confounding effects of the disease state and concomitant medications.

[redacted]

3.4.1. Dose Interval

Following thorough review of all available non-clinical data (pharmacological and toxicological), dosing at each dose level in Part A will be such that 2 subjects (1 AM1476 and 1 placebo) will be dosed at least 24 hours before the remaining 6 subjects. After dosing the first 2 subjects on a separate day, a minimum of a 5-minute dosing interval for the remaining 6 subjects in each dose-ascending group is considered acceptable. Continuation to dose the remaining 6 subjects will be at the Investigator's discretion following review of the data from the first cohort and if it is considered safe to proceed with dosing.

3.5. Selection of Doses in the Study

3.5.1. Starting Dose of 1 mg

In the 13-week repeat-dose toxicology studies, NOAELs in the mouse and dog were 180 mg/kg and 30 mg/kg, respectively. These correspond to human equivalent doses (HEDs) of:

Mouse: 180 mg/kg × 0.081 = 14.58 mg/kg
(equivalent to approximately 875 mg in a 60-kg subject)

Dog: 30 mg/kg × 0.541 = 16.23 mg/kg
(equivalent to approximately 974 mg in a 60-kg subject)

Where 0.081 and 0.541 are conversion factors to extrapolate the animal dose to the HED based on body surface area.[6]

The mouse was the most sensitive species (ie, that with the lowest HED), and with a 10-fold safety margin, this equates to a maximum recommended starting dose of 87.5 mg in a 60-kg subject.

For Part A of the study, the starting dose is 1 mg.

Two physiologically based PK models have been developed to project the PK following oral administration in humans. Based on the more conservative of the models the starting dose of 1 mg is expected to result in the exposure and respective inhibition of the 5-HT_{2B} receptor as defined in Table 3.

Table 3: Predicted Inhibition of the 5-HT_{2B} Receptor at a Single Dose of 1 mg AM1476

	1 mg AM1476
AUC_{0-24} (ng·h/mL)	8.30
C_{max} (ng/mL)	0.63
Inhibition over 24 hours (%)	28.6
Inhibition at peak exposure (%)	37.9
Duration of inhibition > 30% (hours)	7
Duration of inhibition > 20% (hours)	24
Duration of inhibition > 10% (hours)	24

Abbreviations: AUC_{0-24} = area under the plasma concentration-time curve from time zero to 24 hours postdose; C_{max} = maximum observed plasma concentration.
Data based on physiologically based pharmacokinetic (PBPK) models using GastroPlus software. The model utilised physiologically based in vitro to in vivo scaling of hepatic clearance without empirical scaling, consistent with the assumption that in vitro hepatocyte data predicts in vivo clearance equally well in mouse and in human.

Bioavailability, but not protein binding, have been taken into account when using the model (Table 3). This is considered reasonable as there are no signs of acute toxicity following exposure with AM1476 in mice or dogs in the toxicology studies.

Based on these predictions, exposure following a 1 mg dose is expected to be 1135- and 2206-fold below the exposure observed at the NOAEL in the mouse based on AUC_{0-24} and C_{max} (ie, at the NOAEL with the lowest HED in the female mouse), respectively.

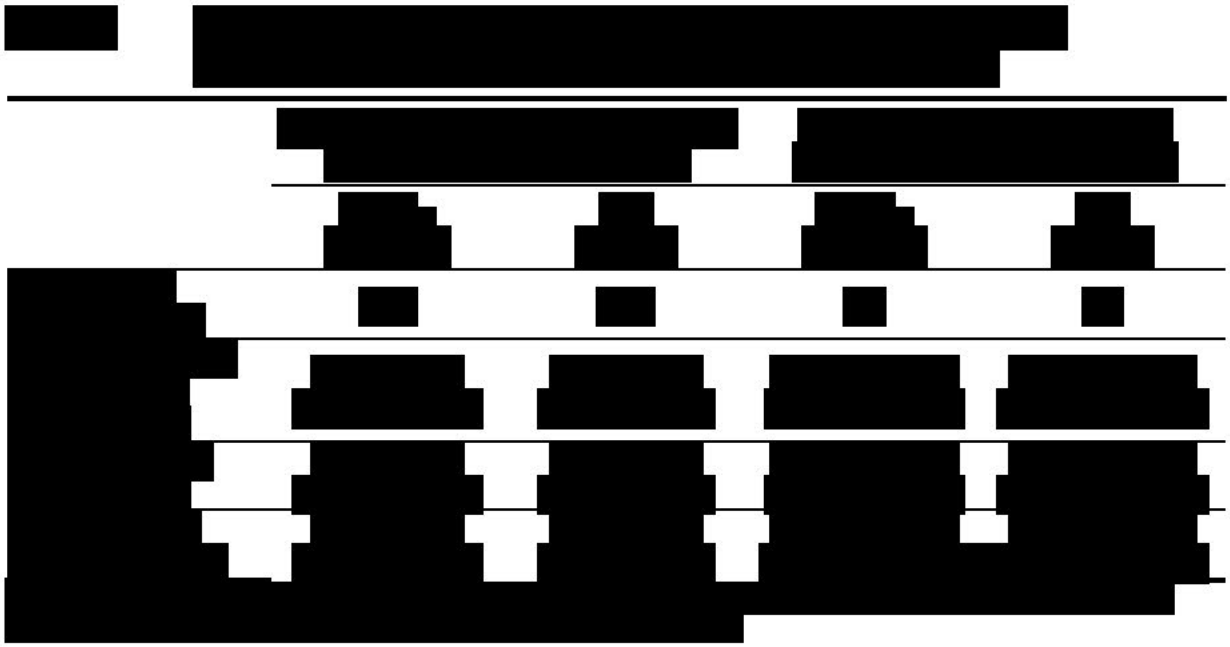
In vivo, oral doses of 1 mg/kg in the mouse were shown to be pharmacologically active, although 1 mg/kg is considered sub-therapeutic. This corresponds to a HED of 0.081 mg/kg (equivalent to approximately 4.8 mg in a 60-kg subject).

Based on in vivo pharmacology studies, a starting dose of 1 mg is considered to have a minimal anticipated pharmacological effect as the starting dose is 4.8-fold lower than the lowest pharmacologically active dose established in 2 different rodent models.

It should further be noted that this is not the first time humans will be exposed to a 5-HT_{2B} receptor antagonist. The non-selective compound, terguride, inhibiting a range of receptors, among them the 5-HT_{2B} receptor, is approved for use in humans in some markets and has undergone clinical studies in Europe.[7,8]

3.5.2. Maximum Dose

The safety margins (based on exposure) for the relevant non-clinical findings at the maximum permitted exposure based on NOAEL in the mouse following repeated dosing for 85 days are presented in Table 5.



The proposed IMP dose levels for Part A and Part B are shown in Table 6. However, based on the ongoing review of the available safety, tolerability, and PK data, as applicable, doses may be adjusted.

The dose levels for Part A will be decided in consultation with the Sponsor, on the basis of available safety, tolerability, and PK data (as applicable) from lower doses and may be adjusted based on the ongoing review of these data. For Part B of the study, dose levels, dosing frequency, and dosing duration will be decided, in consultation with the Sponsor, on the basis of data from Part A and earlier groups in Part B. The total daily exposure of AM1476 administered during this part of the study will be planned to not exceed an exposure shown to be safe and well tolerated in Part A.

Although dosing is planned to be QD on Days 1 to 10 (inclusive) in Part B, the dosing frequency, the number of days of dosing, and dietary state (including fasting requirement and meal compositions), may be changed following review of data from Parts A and B, as applicable. The dose regimen will comprise no less than once every 2 days and will not exceed BID dosing and the dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days.

Table 6: Proposed Investigational Medicinal Product Dose Levels for Parts A and B

Study Part	Group	Subject Numbers	Treatment Period 1	Treatment Period 2
Part A	A1	0101 – 0108	1 mg or placebo	NA
	A2	0109 – 0116	TBD mg or placebo	NA
	A3[a]	0117 – 0124	TBD mg (fasted) or placebo	TBD mg (fed) or placebo
	A4	0125 – 0132	TBD mg or placebo	NA
	A5	0133 – 0140	TBD mg or placebo	NA
	A6	0141 – 0148	TBD mg or placebo	NA
	A7[b]	0149 – 0156	TBD	TBD
	A8[b]	0157 – 0164	TBD	TBD
	A9[b]	0165 – 0172	TBD	TBD
Part B	B1	0201 – 0208	TBD mg or placebo	NA
	B2	0209 – 0216	TBD mg or placebo	NA
	B3	0217 – 0224	TBD mg or placebo	NA
	B4[b]	0225 – 0232	TBD	TBD
	B5[b]	0233 – 0240	TBD	TBD
	B6[b]	0241 – 0248	TBD	TBD

Abbreviations: NA = not applicable; PK = pharmacokinetic(s); TBD = to be determined.
a. Although a food-effect evaluation is planned to occur in Group A3, this may be subject to change depending upon ongoing review of the PK data.
b. Following review of the safety, tolerability, and PK data, additional dose groups (where systemic exposure is not expected to exceed that stated in the dose escalation stopping criteria [Section 3.7]) may be added to the study. Up to 3 further groups of 8 subjects (6 active:2 placebo) may be included in each of Parts A and B.

For predicted non-pharmacologically active dose levels, dose levels should not increase such that the maximum exposure (as assessed by C_{max} and/or AUC_{0-24}) is predicted to increase by > 5-fold for any individual subject. For predicted pharmacologically active dose levels, dose levels should not increase such that the maximum exposure (as assessed by C_{max} and/or AUC_{0-24}) is predicted to increase by > 3-fold for any individual subject. Exposures will be planned to not exceed that stated in the dose escalation stopping criteria (Section 3.7). Exposure will be assumed to increase in a dose-proportional manner until there is sufficient PK data available to suggest PK is not linear.

Details of all doses administered in Parts A and B of the study will be documented in the TMF.

3.6. Dose Escalation

Doses will be administered in an escalating manner following satisfactory review by the Sponsor and Investigator of the safety and tolerability data (up to 48 hours post-final dose) and plasma PK data (up to 24 hours post-final dose [ie, Day 1 in Part A and Day 10 in Part B]) from the lower dose levels. Doses may be reduced and may be lower than the starting dose. There will be a minimum of 6 days between dose escalations to allow sufficient time for an adequate safety review.

Dose escalation in both Parts A and B will only occur if data from a minimum of 6 subjects have been reviewed from the previous lower dose group, such that data from a minimum of 4 subjects who have received AM1476 will be used to make the dose escalation decision.

The justification for this is as follows:

- The study treatment is of a known pharmacological class for which the on-target effects in humans are well described in different diseases. Based upon non-clinical data, no clinically important off-target effects are expected within the proposed dose range.
- A minimum of 4 subjects receiving the active drug is considered sufficient to characterise the safety profile and PK response to AM1476.

Between each dose escalation, the Investigator will review available data in a blinded manner to ensure it is safe to proceed with the planned dose escalation. An interim safety report, summarising results from available safety assessments, will be sent to the Sponsor prior to the start of each successive group/treatment period. Any clinically significant results will be discussed with the Sponsor before dose escalation continues. Interim PK data will also be reviewed in terms of dose escalation and to confirm that the study design remains appropriate. In the event of a disagreement between Sponsor and Investigator on the dose escalation decision, the decision of the Investigator will be upheld.

For predicted non-pharmacologically active dose levels, dose levels should not increase such that the maximum exposure (as assessed by C_{max} and/or AUC_{0-24}) is predicted to increase by > 5-fold for any individual subject. For predicted pharmacologically active dose levels, dose levels should not increase such that the maximum exposure (as assessed by C_{max} and/or AUC_{0-24}) is predicted to increase by > 3-fold for any individual subject. Exposures will be planned to not exceed that stated in the dose escalation stopping criteria (Section 3.7). Exposure will be assumed to increase in a dose-proportional manner until there is sufficient PK data available to suggest PK is not linear.

3.7. Dose Escalation Stopping Criteria

The study will be halted if 1 or more subjects experience a serious adverse event (SAE) that is considered to be related to IMP or 2 or more subjects in the same group experience severe AEs that are considered to be related to IMP. If, following an internal safety review, the Sponsor deems it appropriate to restart the study, this can be done following approval of a substantial protocol amendment.

Dosing of subjects, including any ongoing multiple dose groups, will be stopped immediately if any of the dose escalation stopping criteria (Section 3.7) are met.

[redacted]

4. SELECTION OF STUDY POPULATION

4.1. Inclusion Criteria

Subjects must satisfy all of the following criteria at the Screening visit unless otherwise stated:

1. Males or females, of any race, between 18 and 60 years of age, inclusive.
2. A body mass index (BMI) between 18.0 and 32.0 kg/m^2, inclusive.
3. In good health, determined by no clinically significant findings from medical history, physical examination, 12-lead ECG, vital signs measurements, and clinical laboratory evaluations (congenital non-haemolytic hyperbilirubinemia [eg, suspicion of Gilbert's syndrome based on total and direct bilirubin] is not acceptable) at Screening and/or Check-in (Day -1) as assessed by the Investigator (or designee).
4. Females will not be pregnant or lactating, and females of childbearing potential and males will agree to use contraception as detailed in Appendix 4.
5. Able to comprehend and willing to sign an ICF and to abide by the study restrictions.

4.2. Exclusion Criteria

Subjects will be excluded from the study if they satisfy any of the following criteria at the Screening visit unless otherwise stated:

1. Significant history or clinical manifestation of any metabolic, allergic, dermatological, hepatic, renal, haematological, pulmonary, cardiovascular, gastrointestinal, neurological, respiratory, endocrine, or psychiatric disorder, as determined by the Investigator (or designee).

2. History of significant hypersensitivity, intolerance, or allergy to any drug compound, food, or other substance, unless approved by the Investigator (or designee).
3. History of stomach or intestinal surgery or resection that would potentially alter absorption and/or excretion of orally administered drugs (uncomplicated appendectomy and hernia repair will be allowed).
4. Any of the following observed in at least 2 of 3 ECG measurements performed:
    a. QTcF > 450 msec.
    b. QRS duration > 110 msec.
    c. PR interval > 220 msec.
    d. findings which would make QTc measurements difficult or QTc data uninterpretable.
5. Any history of additional risk factors for torsades de pointes (eg, heart failure, hypokalaemia, family history of long QT syndrome).
6. Any history or current controlled or uncontrolled hypertension or systolic blood pressure > 140 mmHg or diastolic blood pressure > 90 mmHg confirmed by repeat measurement.
7. History of alcoholism or drug/chemical abuse within 2 years prior to Check-in (Day -1).
8. Alcohol consumption of > 21 units per week for males and > 14 units per week for females. One unit of alcohol equals ½ pint (285 mL) of beer or lager, 1 glass (125 mL) of wine, or 1/6 gill (25 mL) of spirits.
9. Positive alcohol breath test result or positive urine drug screen (confirmed by repeat) at Screening or Check-in (Day -1).
10. Positive hepatitis panel and/or positive human immunodeficiency virus test (Appendix 2).
11. Participation in a clinical study involving administration of an investigational drug (new chemical entity) in the past 90 days prior to dosing.
12. Use or intend to use any medications/products known to alter drug absorption, metabolism, or elimination processes, including St. John's Wort, within 30 days prior to dosing, unless deemed acceptable by the Investigator (or designee).
13. Use or intend to use any prescription medications/products other than hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives within 14 days prior to dosing, unless deemed acceptable by the Investigator (or designee).
14. Use or intend to use slow release medications/products considered to still be active within 14 days prior to Check-in (Day -1), unless deemed acceptable by the Investigator (or designee).
15. Use or intend to use any non-prescription medications/products including vitamins, minerals, and phytotherapeutic/herbal/plant-derived preparations within 7 days prior to Check-in (Day -1), unless deemed acceptable by the Investigator (or designee).

16. Use of tobacco- or nicotine-containing products within 3 months prior to Check-in (Day -1) or positive cotinine at Screening or Check-in (Day -1).
17. Ingestion of poppy seeds, Seville orange, or grapefruit-containing foods or beverages within 7 days prior to Check-in (Day -1).
18. Subjects who are vegetarians, vegans, or are unable to consume the high-fat breakfast (subjects who will participate in a food-effect evaluation [planned to be Group A3] only).
19. Receipt of blood products within 2 months prior to Check-in (Day -1).
20. Donation of blood from 3 months prior to Screening, plasma from 2 weeks prior to Screening, or platelets from 6 weeks prior to Screening.
21. Poor peripheral venous access.
22. Have previously completed or withdrawn from this study or any other study investigating AM1476, and have previously received AM1476.
23. Subjects who are not willing to minimise or avoid exposure to natural or artificial sunlight (tanning beds or UVA/B treatment) following administration of study drug until 2 weeks after the last dose.
24. Subjects who, in the opinion of the Investigator (or designee), should not participate in this study.

Subjects may previously have been screened on a generic basis to determine their eligibility for inclusion in Phase I clinical studies conducted at the CRU. If generic screening was performed within the specified study screening window, selected study-specific procedures will be repeated either at an additional Screening visit or on admission to the CRU on Day -1.

4.3. Rescreening of Subjects

Subjects who are not eligible for inclusion in the study may not be rescreened. Subjects who are eligible for inclusion in the study, but are not dosed within 28 days of Screening, may subsequently be included in the study providing they continue to meet the criteria for inclusion in the study during rescreening.

4.4. Subject Number and Identification

Subjects will have a unique identification number used at Screening. Subjects will be assigned a subject number at the time of their randomisation. Assignment of subject numbers will be in ascending order in each part and no numbers will be omitted (eg, in Part A Subjects 0101, 0102, etc, in Part B Subjects 0201, 0202, etc).

Replacement subjects (Section 4.5) will be assigned a subject number corresponding to the number of the subject he/she is replacing plus 1000 (eg, Subject 1101 replaces Subject 0101).

Subjects will be identified by Screening identification number or subject number only on all study documentation. A list identifying the subjects by subject number will be kept in the Site Master File (SMF).

4.5. Subject Withdrawal and Replacement

A subject is free to withdraw from the study at any time. In addition, a subject will be withdrawn if any of the following criteria are met:

- change in compliance with any inclusion/exclusion criterion that is clinically relevant and affects subject safety as determined by the Investigator (or designee).
- non-compliance with the study restrictions that might affect subject safety or study assessments/objectives, as considered applicable by the Investigator (or designee).
- any clinically relevant sign or symptom that, in the opinion of the Investigator (or designee), warrants subject withdrawal.
- evidence of clinically significant increases in liver function tests defined as 3 times the ULN for AST, ALT, ALP, or GGT or 2 times the ULN for total bilirubin (confirmed with repeat testing).
- [redacted]
- severe hypertension (systolic blood pressure > 180 mmHg and/or diastolic blood pressure > 110 mmHg) in the resting supine position (confirmed by 2 repeat measurements) on at least 2 occasions within a 24-hour interval.
- moderate nausea or vomiting that prevents a subject from eating a meal on 3 or more occasions on 2 consecutive days.

If a subject is withdrawn, the Sponsor will be notified and the date and reason(s) for the withdrawal will be documented in the subject's electronic Case Report Form (eCRF). If a subject is withdrawn, efforts will be made to perform all follow-up assessments, if possible (Appendix 6 for Part A and Appendix 7 for Part B). Other procedures may be performed at the Investigator's (or designee's) and/or Sponsor's discretion. If the subject is in-house, these procedures should be performed before the subject is discharged from the clinic. The Investigator (or designee) may also request that the subject return for an additional Follow-up visit. All withdrawn subjects will be followed until resolution of all their AEs or until the unresolved AEs are judged by the Investigator (or designee) to have stabilised.

Subjects who are withdrawn for reasons not related to study treatment may be replaced following discussion between the Investigator and the Sponsor. Subjects withdrawn as a result of AEs thought to be related to the study treatment will generally not be replaced.

4.6. Study Termination

The study may be discontinued at the discretion of the Investigator (or designee), Sponsor, or Sponsor's Medical Monitor if any of the following criteria are met:

- AEs unknown to date (ie, not previously reported in any similar investigational study drug trial with respect to their nature, severity, and/or duration)
- increased frequency, severity, and/or duration of known, anticipated, or previously reported AEs (this may also apply to AEs defined at Check-in [Day -1] as baseline signs and symptoms)
- medical or ethical reasons affecting the continued performance of the study
- difficulties in the recruitment of subjects
- cancellation of drug development.

5. STUDY TREATMENTS

5.1. Description, Storage, Packaging, and Labelling

Active pharmaceutical ingredient (API; AM1476 powder) will be supplied by the Sponsor, along with the batch/lot number and Certificate of Analysis, Transmissible Spongiform Encephalopathy (TSE) statements, and a declaration of Good Manufacturing Practice (GMP). A Certificate of Release authorised by a Qualified Person in the European Union will also be issued, by Covance CRU Qualified Person, for each batch of IMP prior to administration to subjects.

The Sponsor will supply a sufficient quantity of API for the manufacture of the unit doses at Covance CRU. All excipients will be sourced by Covance.

The API and IMP will be stored according to the instructions on the label at the CRU in a location that is locked with restricted access.

5.2. Study Treatment Administration

Full details on dietary restrictions for dose administration are described in Section 6.2.

Each dose of AM1476 and placebo will be administered orally with approximately 240 mL of room-temperature water. In Part A, all doses will be administered after an overnight fast of at least 10 hours, with the exception of Group A3, where the dose given in Treatment Period 2 will be administered 30 minutes after starting a high-fat breakfast. The dietary status for dosing in Part B will be determined following review of the PK data from the fed/fasted comparison in Part A.

Subjects will be administered the IMP in numerical order while standing and will not be permitted to lie supine for 2 hours after dosing, except as necessitated by the occurrence of an AE(s) and/or study procedures.

5.3. Randomisation

The randomisation code will be produced by the statistics department at Covance using a computer-generated pseudo-random permutation procedure.

In Part A, 6 subjects per group will be randomly assigned to receive AM1476 and 2 subjects per group will be randomly assigned to receive placebo, and subjects in Group A3 will receive the same treatment in Treatment Periods 1 and 2. For all groups in Part A, sentinel dosing will occur whereby 2 subjects (1 active and 1 placebo) will be dosed on 1 day and, providing no safety concerns arise, the remaining 6 subjects (5 active and 1 placebo) will be dosed after at least 24 hours.

In Part B, 6 subjects will be randomly assigned to receive AM1476 and 2 subjects per group will be randomly assigned to receive placebo.

5.4. Blinding

The following controls will be employed to maintain the double-blind status of the study:

- The placebo capsules will be identical in appearance to the AM1476.
- The Investigator and other members of staff (with the exception of pharmacy staff) involved with the study will remain blinded to the treatment randomisation code during the conduct of the study.
- Interim bioanalytical data will be provided to Covance in a blinded manner.

To maintain the blind, the Investigator will be provided with a sealed randomisation code for each subject, containing details of their treatment. These individual sealed envelopes will be kept in a limited access area that is accessible 24 hours a day. In order to manage subject safety or to support dose escalation decisions (in the event of possibly treatment-related SAEs or severe AEs), the decision to unblind resides solely with the Investigator. Whenever possible, and providing it does not interfere with or delay any decision in the best interest of the subject, the Investigator will discuss the intended code-break with the Sponsor. If it becomes necessary to break the code during the study, the date, time, and reason will be recorded in the subject's source data and on the individual envelope and will be witnessed by a second person.

5.5. Treatment Compliance

The following measures will be employed to ensure treatment compliance:

- All doses will be administered under the supervision of suitably qualified study site staff.
- Immediately after dose administration, visual inspection of the mouth and hands will be performed for each subject.
- At each dosing occasion, a predose and postdose inventory of IMP will be performed on the dose containers.

5.6. Drug Accountability

The Investigator (or designee) will maintain an accurate record of the receipt of the study supplies received, dispensed, and destroyed. In addition, an accurate drug disposition record will be kept, specifying the amount dispensed to each subject and the date of dispensing. This drug accountability record will be available for inspection at any time. At the completion of the study, the original drug accountability record will be available for review by the Sponsor upon request.

For each batch of unit doses, the empty used unit dose containers will be discarded upon satisfactory completion of the compliance and accountability procedures. Any unused assembled unit doses will be retained until completion of the study.

At the completion of the study, all unused supplies will be returned to the Sponsor or disposed of by the study site, per the Sponsor's written instructions.

6. CONCOMITANT THERAPIES AND OTHER RESTRICTIONS

6.1. Concomitant Therapies

Subjects will refrain from use of any prescription or non-prescription medications/products during the study until the Follow-up visit, unless the Investigator (or designee) and/or Sponsor have given their prior consent.

Paracetamol/acetaminophen (2 g/day for up to 3 consecutive days), hormone replacement therapy, oral, implantable, transdermal, injectable, or intrauterine contraceptives are acceptable concomitant medications. The administration of any other concomitant medications during the study is prohibited without prior approval of the Investigator (or designee), unless its use is deemed necessary for treatment of an AE. Any medication taken by a subject during the course of the study and the reason for its use will be documented in the source data.

6.2. Diet

While confined at the study site, subjects will receive a standardised diet at scheduled times that do not conflict with other study-related activities. Subjects will be fasted (at least 8 hours) before collection of blood samples for clinical laboratory evaluations.

On the days with intensive PK assessments (Day 1 for Part A, and Days 1 and 10 for Part B), meals will be identical for each group with the exception of the high-fat breakfast for Group A3.

On Day 1 in Part A, subjects will be fasted for at least 10 hours prior to dosing until approximately 4.5 hours after dosing, when lunch will be provided. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to until 2 hours after dosing. Meals will be provided as appropriate at other times. Other than the fluid restrictions on dosing days, water will be freely available at all times.

Subjects in Group A3 in Treatment Period 2 will consume a high-fat breakfast (contents are detailed in Table 7) before dosing. Subjects should start the meal 30 minutes prior to

administration of the IMP. Study subjects should eat this meal in 30 minutes or less. The drug product should be administered 30 minutes after start of the meal. Subjects will be fasted for approximately 4.5 hours after dosing and will not be allowed fluids until 2 hours after dosing.

Table 7: High-fat Breakfast Content

High-fat Breakfast
120 g fried eggs (2 eggs) in vegetable oil
50 g bacon (2 rashers)
72 g toasted white bread (2 slices)
13 g butter (2 pats)
108 g hash brown (3 each)
240 g whole milk
Total calories: 973 kcal

This high-fat breakfast contains the equivalent of approximately 150 protein calories, 250 carbohydrate calories, and 500 to 600 fat calories.

In Part B, the time interval between meals and dosing will be determined by the PK data from Part A and will be documented in the TMF. Meals will be provided as appropriate at other times. With the exception of water given with the dose, subjects will not be allowed fluids from 1 hour prior to dosing until 2 hours after dosing. Other than these fluid restrictions, water will be freely available at all times.

Foods and beverages containing poppy seeds, grapefruit, or Seville oranges will not be allowed from 7 days prior to Check-in (Day -1) until the Follow-up visit.

Caffeine-containing foods and beverages will not be allowed from 36 hours before Check-in (Day -1) until Discharge.

Consumption of alcohol will not be permitted from 36 hours prior to Check-in (Day -1) until Discharge. Up to 2 units/day of alcohol are permitted from Discharge until 36 hours before the Follow-up visit or 36 hours before Check-in (Day -1) in each treatment period.

6.3. Smoking

Subjects will not be permitted to use tobacco- or nicotine-containing products within 3 months prior to Check-in (Day -1) until the Follow-up visit.

6.4. Exercise

Subjects are required to refrain from strenuous exercise from 7 days before Check-in (Day -1) until the Follow-up visit and will otherwise maintain their normal level of physical activity during this time (ie, will not begin a new exercise program nor participate in any unusually strenuous physical exertion).

6.5. Blood Donation

Subjects are required to refrain from donation of blood from 3 months prior to Screening, plasma from 2 weeks prior to Screening, and platelets from 6 weeks prior to Screening until 3 months after the Follow-up visit.

6.6. Exposure to Ultraviolet Light

Subjects should minimise or avoid exposure to natural or artificial sunlight (tanning beds or UVA/B treatment) following administration of study drug until 2 weeks after the last dose. If subjects need to be outdoors during this period, they should wear loose fitting clothes that protect skin from sun exposure and discuss other sun protection measures with the Investigator.

7. STUDY ASSESSMENTS AND PROCEDURES

Study assessments and procedures will be performed at the timepoints indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B.

Every effort will be made to schedule and perform the procedures as closely as possible to the nominal time, giving considerations to appropriate posture conditions, practical restrictions, and the other procedures to be performed at the same timepoint.

The highest priority procedures will be performed closest to the nominal time. The order of priority for scheduling procedures around a timepoint is (in descending order of priority):

- dosing
- blood samples (for AM1476 assay)
- urine samples (for AM1476 assay)
- any other procedures.

Where activities at a given timepoint coincide, consideration must be given to ensure that vital signs and single 12-lead ECGs are measured and [redacted] occurs before blood draws.

7.1. Pharmacokinetic Assessments

7.1.1. Sample Collection and Processing

Blood samples (approximately 1 × 3.0 mL) will be collected by venepuncture or cannulation at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. Furthermore, up to 3 additional blood samples may be taken from each subject per treatment period, with the maximum volume of blood withdrawn per subject not exceeding the limit detailed in Appendix 3. Any changes to the scheduled times of PK assessments will be agreed with the Sponsor and documented in the TMF. Samples taken from subjects who received placebo will not be analysed.

Procedures for collection, processing, and shipping of PK blood samples will be detailed in a separate document.

Urine samples will be collected into pre-weighed polyethylene containers over the time intervals indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. Procedures for collection, processing, and shipping of urine samples will be detailed in a separate document.

[redacted]

7.1.2. Analytical Methodology

Plasma and urine concentrations of AM1476 will be determined using validated analytical procedures. Specifics of the analytical methods will be provided in separate documents.

[redacted]

7.2. Safety and Tolerability Assessments

7.2.1. Adverse Events

Adverse event definitions, assignment of severity and causality, and procedures for reporting SAEs are detailed in Appendix 1.

The condition of each subject will be monitored from the time of signing the ICF to final discharge from the study. Subjects will be observed for any signs or symptoms and asked about their condition by open questioning, such as "How have you been feeling since you were last asked?", at least once each day while resident at the study site and at each study visit. Subjects will also be encouraged to spontaneously report AEs occurring at any other time during the study.

Any AEs and remedial action required will be recorded in the subject's source data. The nature, time of onset, duration, and severity will be documented, together with an Investigator's (or designee's) opinion of the relationship to study treatment.

Adverse events recorded during the course of the study will be followed up, where possible, until resolution or until the unresolved AEs are judged by the Investigator (or designee) to have stabilised. This will be completed at the Investigator's (or designee's) discretion.

7.2.2. Clinical Laboratory Evaluations

Blood and urine samples will be collected for clinical laboratory evaluations at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. Clinical laboratory evaluations are listed in Appendix 2. The maximum volume of blood withdrawn per subject will not exceed the limit detailed in Appendix 3. Additional clinical laboratory evaluations will be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of clinical laboratory safety evaluations is required.

Subjects will be asked to provide urine samples for drugs of abuse screen and cotinine test, and will undergo an alcohol breath test at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. For all female subjects, a pregnancy test

will be performed at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B.

An Investigator (or designee) will perform a clinical assessment of all clinical laboratory data.

7.2.3. Vital Signs

Supine blood pressure, supine pulse rate, respiratory rate, and oral body temperature will be assessed at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. Vital signs may also be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of vital signs is required.

Day 1 predose blood pressure, pulse rate, and respiratory rate will be measured in triplicate at approximately 2-minute intervals. The median value will be used as the baseline value in the data analysis. Oral body temperature will be measured singly. All subsequent measurements will be performed singly and repeated once if outside the relevant clinical reference ranges.

Subjects must be supine for at least 5 minutes before blood pressure and pulse rate measurements.

For orthostatic vital sign measurements, the supine blood pressure and pulse rate will be measured after the subject has been supine for at least 5 minutes. The subject will then stand for at least 2 minutes and the standing blood pressure and pulse rate will be measured.

7.2.4. Electrocardiograms

7.2.4.1. 12-lead Electrocardiograms

Resting 12-lead ECGs will be recorded after the subject has been supine and at rest for at least 5 minutes at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B. All scheduled 12-lead ECGs will be measured in triplicate at approximately 1-minute intervals. The mean values at each timepoint will be used in the data analysis.

Additional 12-lead ECGs may be performed at other times if judged to be clinically appropriate or if the ongoing review of the data suggests a more detailed assessment of ECGs is required. The Investigator (or designee) will perform a clinical assessment of each 12-lead ECG.

7.2.4.2. Telemetry

Cardiac rhythm will be monitored by telemetry at the times indicated in the Schedule of Assessments in Appendix 6 for Part A.

Telemetry is not planned for Part B of the study but may be implemented if any clinically significant findings are identified in the data from Part A.

7.2.5. Physical Examination

A full physical examination or symptom-directed physical examination will be performed at the timepoints specified in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B.

7.2.6. Neurological Examination

A neurological examination (scheduled or symptom-directed) will be performed at the timepoints specified in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B.

The neurological examination will include an assessment of mental status, cranial nerves, motor system, reflexes, and cerebellar function.

7.2.7. Columbia-Suicide Severity Rating Scale

A C-SSRS questionnaire will be administered in Part B at the times indicated in the Schedule of Assessments in Appendix 7.

The C-SSRS has 5 questions that address suicidal ideation, 5 sub-questions that are used to assess the intensity of ideation and 4 questions that address suicidal behaviour.

The Baseline (Lifetime) C-SSRS will be utilised at Screening and the Since Last Visit C-SSRS will be utilised at all other visits.

7.2.8. Body Weight

Body weight (in underclothes) will be recorded at the times indicated in the Schedule of Assessments in Appendix 6 for Part A and Appendix 7 for Part B.

[redacted]

8. SAMPLE SIZE AND DATA ANALYSIS

8.1. Determination of Sample Size

No formal statistical assessment, in terms of sample size, has been conducted as this is the first time AM1476 is being administered to humans. However, the number of subjects in each part of the present study is common in early clinical pharmacology studies and is considered sufficient to achieve the objectives of the study.

8.2. Analysis Populations

All protocol deviations that occur during the study will be considered prior to database lock for their severity/impact and will be taken into consideration when subjects are assigned to the appropriate population.

8.2.1. Pharmacokinetic Population

The PK population will include all subjects who received at least 1 dose of AM1476 and have evaluable PK data. A subject will be excluded from the PK summary statistics and statistical analysis if the subject has an AE of vomiting that occurs at or before 2-times median time to maximum concentration or has a protocol deviation considered to affect the assessment of PK.

8.2.2. Safety Population

The safety population will include all subjects who received at least 1 dose of study treatment (AM1476 or placebo).

8.3. Pharmacokinetic Analyses

Non-compartmental PK analysis will be performed on individual plasma and urine concentration data, using commercial software such as Phoenix® WinNonlin®. Individual plasma and urine concentrations of AM1476 and plasma and urine PK parameters will be listed and summarised using descriptive statistics. Individual and mean AM1476 concentration-time profiles will also be presented graphically.

In Part A, where data are available, AM1476 dose proportionality will be examined across dose groups. The PK parameters, $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max}, will be analysed for dose proportionality using a power model approach or analysis of variance (ANOVA) model as appropriate. Where data are available, the effect of food at 1 dose level in Part A will be investigated using ANOVA. To investigate the effect of food on the PK of AM1476, the log-transformed $AUC_{0-\infty}$, $AUC_{0-tlast}$, and C_{max} values for AM1476 administered with and without food will be analysed using a linear mixed-effects model with treatment as fixed effect and subject as a random effect. Point estimates and 90% confidence intervals (CIs) for differences between the treatments (fed versus fasted) on the log scale will be exponentiated to obtain estimates for the ratios of geometric means and respective 90% CIs on the original scale.

In Part B, $AUC_{0-\tau}$ and C_{max} on Day 10 will be analysed for dose proportionality using a power model approach or ANOVA model as appropriate.

Additional details will be presented in the Statistical Analysis Plan (SAP).

8.4. Safety Analysis

Safety parameters will be listed and summarised using descriptive statistics. No formal statistical analysis of safety data is planned. Each AE will be coded using the Medical Dictionary for Regulatory Activities.

8.5. Interim Analysis

No formal interim analyses are planned for this study.

9. REFERENCES

1. Mann DA, Oakley F. Serotonin paracrine signaling in tissue fibrosis. Biochim Biophys Acta. 2013 Jul;1832(7):905–10.

2. Dees C, Akhmetshina A, Zerr P, Reich N, Palumbo K, Horn A, et al. Platelet-derived serotonin links vascular disease and tissue fibrosis. J Exp Med. 2011 May 9;208(5):961–72.

3. Königshoff M, Dumitrascu R, Udalov S, Amarie OV, Reiter R, Grimminger F, et al. Increased expression of 5-hydroxytryptamine2A/B receptors in idiopathic pulmonary fibrosis: a rationale for therapeutic intervention. Thorax. 2010 Nov;65(11):949–55.

4. Rosenbloom J, Macarak E, Piera-Velazquez S, Jimenez SA. Human Fibrotic Diseases: Current Challenges in Fibrosis Research. Methods Mol Biol. 2017;1627:1–23.

5. Distler JHW, Györfi A-H, Ramanujam M, Whitfield ML, Königshoff M, Lafyatis R. Shared and distinct mechanisms of fibrosis. Nat Rev Rheumatol. 2019 Dec;15(12):705–30.

6. AnaMar AB. AM1476 – Investigator's Brochure. (Version 2). 27 August 2020.

7. The Serotonin Receptor 2 Inhibitor Terguride Has Beneficial Effects on Skin Fibrosis: Results from a Phase 2 Proof of Concept Study [Internet]. ACR Meeting Abstracts. Available from: https://acrabstracts.org/abstract/the-serotonin-receptor-2-inhibitor-terguride-has-beneficial-effects-on-skin-fibrosis-results-from-a-phase-2-proof-of-concept-study/

8. Distler O, Eich W, Dokoupilova E, Dvorak Z, Fleck M, Gaubitz M, et al. Evaluation of the efficacy and safety of terguride in patients with fibromyalgia syndrome: Results of a twelve-week, multicenter, randomized, double-blind, placebo-controlled, parallel-group study. Arthritis & Rheumatism. 2010;62(1):291–300.

10. APPENDICES

Appendix 1: Adverse Event Reporting

Definitions

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product, which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and/or unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a study treatment, whether or not related to the study treatment.

Assessment of Severity

The Investigator will be asked to provide an assessment of the severity of the AE using the following categories:

- **Mild:** Usually transient and may require only minimal treatment or therapeutic intervention. The event does not generally interfere with usual activities of daily living.
- **Moderate:** Usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
- **Severe:** Interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention.

Relationship to Study Treatment

The Investigator (or designee) will make a determination of the relationship of the AE to the study treatment using a 4-category system according to the following guidelines:

- **Not Related:** The AE is definitely caused by the subject's clinical state or the study procedure/conditions.
- **Unlikely Related:** The temporal association between the AE and the drug is such that the drug is not likely to have any reasonable association with the AE.
- **Possibly Related:** The AE follows a reasonable temporal sequence from the time of drug administration but could have been produced by the subject's clinical state or the study procedures/conditions.
- **Related:** The AE follows a reasonable temporal sequence from administration of the drug, abates upon discontinuation of the drug, follows a known or hypothesised cause-effect relationship, and (if appropriate) reappears when the drug is reintroduced.

Follow-up of Adverse Events

Every reasonable effort will be made to follow-up with subjects who have AEs. Any subject who has an ongoing AE that is possibly related or related to the investigational medicinal product (IMP) or study procedures at the Follow-up visit will be followed up, where possible,

until resolution or until the unresolved AE is judged by the Investigator (or designee) to have stabilised. This will be completed at the Investigator's (or designee's) discretion. Any subject who has an ongoing AE that is not related or unlikely related to the IMP or study procedures at the Follow-up visit can be closed out as ongoing at the Investigator's discretion.

Adverse Drug Reactions

All noxious and unintended responses to an IMP (ie, where a causal relationship between an IMP and an AE is at least a reasonable possibility) related to any dose should be considered adverse drug reactions.

For marketed medicinal products, a response to a drug which is noxious and unintended and which occurs at doses normally used in man for prophylaxis, diagnosis, or therapy of diseases or for modification of physiological function is to be considered an adverse drug reaction.

An unexpected adverse drug reaction is defined as an adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg, Investigator's Brochure [IB] for an unapproved IMP).

Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose either:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity (disability is defined as a substantial disruption of a person's ability to conduct normal life functions)
- results in a congenital anomaly/birth defect
- results in an important medical event (see below).

Important medical events that may not result in death, be life-threatening, or require hospitalisation may be considered SAEs when, based upon appropriate medical judgment, they may jeopardise the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

Instances of death or congenital abnormality, if brought to the attention of the Investigator at any time after cessation of the study treatment and considered by the Investigator to be possibly related to the study treatment, will be reported to the Sponsor.

<u>Definition of Life-threatening</u>

An AE is life-threatening if the subject was at immediate risk of death from the event as it occurred (ie, does not include a reaction that might have caused death if it had occurred in a more serious form). For instance, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening even though drug-induced hepatitis can be fatal.

Definition of Hospitalisation

Adverse events requiring hospitalisation should be considered serious. In general, hospitalisation signifies that the subject has been detained (usually involving an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate at the Clinical Research Unit (CRU). When in doubt as to whether hospitalisation occurred or was necessary, the AE should be considered as serious.

Hospitalisation for elective surgery or routine clinical procedures, which are not the result of an AE, need not be considered AEs and should be recorded on a Clinical Assessment Form and added to the electronic Case Report Form. If anything untoward is reported during the procedure, this must be reported as an AE and either 'serious' or 'non-serious' attributed according to the usual criteria.

Serious Adverse Event Reporting

Covance Drug Safety Services (DSS) Europe, Maidenhead, United Kingdom, are responsible for coordinating the reporting of SAEs in accordance with the European Directive 2001/20/EC.

The Investigator will complete an SAE report form and forward it by facsimile or email to DSS and the Sponsor immediately (within 24 hours) upon becoming aware of an SAE.

The responsibilities of Covance DSS include the following:

- Prepare an AE reporting plan prior to the start of the study. Where this plan differs from the applicable CRU standard operating procedure on SAE reporting, the Safety Management Plan will always take precedence.
- Receive and review SAE report forms from the CRU and inform the Sponsor of the SAE within 1 working day of the initial notification to DSS. Drug Safety Services will delete any information from the SAE report forms that may identify the subject.
- Write case narratives and enter the case into Covance's safety database as defined in the AE reporting plan.
- Produce appropriate reports of all Suspected Unexpected Serious Adverse Reactions and forward to the Ethics Committee, Medicines and Healthcare Products Regulatory Agency, Principal Investigator, and the Sponsor within the timeframes stipulated in the Clinical Trials Directive Guideline (ENTR/CT 3).

The responsibility for reporting SAEs will be transferred to the Sponsor 28 days after the end of the study.

Pregnancy

Pregnancy (maternal or paternal exposure to study treatment) does not meet the definition of an AE. However, to fulfil regulatory requirements any pregnancy should be reported following the SAE process to collect data on the outcome for both mother and foetus.

Appendix 2: Clinical Laboratory Evaluations

Clinical chemistry:	Haematology:	Urinalysis:
Alanine aminotransferase (ALT) Albumin Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) Calcium Chloride Cholesterol Creatinine Direct bilirubin Gamma-glutamyl transferase (GGT) Glucose Inorganic phosphate Potassium Sodium Total bilirubin Total CO_2 Total protein Uric acid	Haematocrit Haemoglobin Mean cell haemoglobin Mean cell haemoglobin concentration Mean cell volume Platelet count Red blood cell (RBC) count White blood cell (WBC) count WBC differential: Basophils Eosinophils Lymphocytes Monocytes Neutrophils	Blood Glucose Ketones pH Protein Specific gravity Urobilinogen Microscopic examination
Serology:[a]	**Drug screen:**	**Coagulation profile**
Anti-hepatitis B surface antibody Hepatitis B surface antigen Hepatitis C antibody Human immunodeficiency (HIV-1 and HIV-2) antibodies and p24 antigen	Including but not limited to: Amphetamines/methamphetamines Barbiturates Benzodiazepines Cocaine (metabolite) Methadone Phencyclidine Opiates Tetrahydrocannabinol/cannabinoids Tricyclic antidepressants Cotinine test	Activated partial thromboplastin time International normalised ratio Prothrombin time
		Hormone panel - females only:
		Follicle-stimulating hormone[a] Serum pregnancy test (human chorionic gonadotropin)[b] Urine pregnancy test[b]

a. Performed at Screening only.
b. For all females, performed in serum at Screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.

Appendix 3: Total Blood Volume

Part A

The following blood volumes will be withdrawn for each subject not participating in a food-effect evaluation:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	4	30.0
Coagulation	1.8	4	7.2
Serology	3.5	1	3.5
[redacted]	5.0	1	5.0
AM1476 PK[b]	3.0	18	54.0
Total:			99.7

Abbreviations: PK = pharmacokinetic(s).
a. Includes pregnancy and follicle-stimulating hormone (FSH) test.
b. Includes an additional 3 blood samples per treatment period (Section 7.1.1). [redacted] No additional blood samples will be taken.

The following blood volumes will be withdrawn for each subject participating in a food-effect evaluation:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	6	45.0
Coagulation	1.8	6	10.8
Serology	3.5	1	3.5
[redacted]	5.0	1	5.0
AM1476 PK[b]	3.0	36	108.0
Total:			172.3

Abbreviations: FSH = follicle-stimulating hormone; PK = pharmacokinetic(s).
a. Includes pregnancy and FSH test.
b. Includes an additional 3 blood samples per treatment period (Section 7.1.1). [redacted] No additional blood samples will be taken.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 150 mL for subjects not participating in a food effect evaluation and 220 mL for subjects participating in a food effect evaluation.

Part B

The following blood volumes will be withdrawn for each subject:

	Volume per blood sample (mL)	Maximum number of blood samples	Total amount of blood (mL)
Clinical laboratory evaluations[a]	7.5	6	45.0
Coagulation	1.8	6	10.8
Serology	3.5	1	3.5
[redacted]	5.0	1	5.0
AM1476 PK[b]	3.0	36	108.0
Total:			172.3

Abbreviations: FSH = follicle-stimulating hormone; PK = pharmacokinetic(s).
a. Includes pregnancy and FSH test.
b. Includes an additional 3 blood samples per treatment period (Section 7.1.1). [redacted] No additional blood samples will be taken.

If extra blood samples are required, the maximum blood volume to be withdrawn per subject will not exceed 220 mL.

Appendix 4: Contraception Guidance

Definitions

Women of Childbearing Potential: premenopausal females who are anatomically and physiologically capable of becoming pregnant following menarche.

Women of Non-childbearing Potential:

1. **Surgically sterile:** females who are permanently sterile via hysterectomy, bilateral salpingectomy, and/or bilateral oophorectomy by reported medical history and/or medical records. Surgical sterilisation to have occurred a minimum of 6 weeks, or at the Investigator's discretion, prior to Screening.
2. **Postmenopausal:** Females at least 45 years of age with amenorrhoea for 12 months without an alternative medical reason with confirmatory follicle-stimulating hormone (FSH) levels of ≥ 40 mIU/mL. The amenorrhoea should not be induced by a medical condition such as anorexia nervosa, hypothyroid disease or polycystic ovarian disease, or by extreme exercise. It should not be due to concomitant medications that may have induced the amenorrhoea such as oral contraceptives, hormones, gonadotropin-releasing hormones, anti-oestrogens, or selective oestrogen receptor modulators. Females on hormone replacement therapy with FSH levels < 40 mIU/mL may be included at the discretion of the Investigator.

Fertile male: a male that is considered fertile after puberty.

Infertile male: permanently sterile male via bilateral orchiectomy.

Contraception Guidance

Female Subjects

Female subjects who are of non-childbearing potential will not be required to use contraception. Female subjects of childbearing potential must be willing to use 2 methods (1 primary highly effective and 1 secondary method) of birth control from the time of signing the Informed Consent Form (ICF) until 90 days after the Follow-up visit. Primary highly effective methods of contraception include:

- surgical method performed at least 3 months prior to the Screening visit:
  - bilateral tubal ligation
  - Essure® (hysteroscopic bilateral tubal occlusion) with confirmation of occlusion of the fallopian tubes
- hormonal implant eg, Implanon (as prescribed)
- hormonal or non-hormonal intrauterine device (IUD) with appropriate re-insertion period (as prescribed)

- vasectomised male partner (sterilisation performed at least 90 days prior to the Screening visit, with verbal confirmation of surgical success, and the sole partner for the female subject).

Secondary (barrier) methods of contraception include:

- male condom with spermicide
- female condom with spermicide
- over-the-counter sponge with spermicide
- cervical cap with spermicide (as prescribed)
- diaphragm with spermicide (as prescribed).

Female subjects can only choose a cervical cap or diaphragm if they have already had a prescription and fitting by their healthcare provider to ensure protocol requirements are met.

Female subjects of childbearing potential should refrain from donation of ova from Check-in (Day -1) until 90 days after the Follow-up visit.

Male Subjects

Male subjects (even with a history of vasectomy) with partners of childbearing potential must use a male barrier method of contraception (ie, male condom with spermicide) in addition to a second method of acceptable contraception from Check-in (Day -1) until 90 days after the Follow-up visit. Acceptable methods of contraception for female partners include:

- hormonal injection
- combined oral contraceptive pill or progestin/progestogen-only pill
- combined hormonal patch
- combined hormonal vaginal ring
- surgical method (bilateral tubal ligation or Essure® [hysteroscopic bilateral tubal occlusion])
- hormonal implant
- hormonal or non-hormonal IUD
- over-the-counter sponge with spermicide
- cervical cap with spermicide
- diaphragm with spermicide.

An acceptable second method of contraception for male subjects is vasectomy that has been performed at least 90 days prior to the Screening visit, with verbal confirmation of surgical success.

For male subjects (even with a history of vasectomy), sexual intercourse with female partners who are pregnant or breastfeeding should be avoided unless condoms are used from the time

of the first dose until 90 days after the Follow-up visit. Male subjects are required to refrain from donation of sperm from Check-in (Day -1) until 90 days after the Follow-up visit.

Sexual Abstinence and Same-sex Relationships

Subjects who practice true abstinence, because of the subject's lifestyle choice (ie, the subject should not become abstinent just for the purpose of study participation), are exempt from contraceptive requirements. Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception. If a subject who is abstinent at the time of signing the ICF becomes sexually active they must agree to use contraception as described previously.

For subjects who are exclusively in same-sex relationships, contraceptive requirements do not apply. If a subject who is in a same-sex relationship at the time of signing the ICF becomes engaged in a heterosexual relationship, they must agree to use contraception as described previously.

Appendix 5: Regulatory, Ethical, and Study Oversight Considerations

Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines.
- Applicable International Conference on Harmonisation (ICH) Good Clinical Practice (GCP) Guidelines.
- Applicable laws and regulations.

The protocol, protocol amendments, Informed Consent Form (ICF), Investigator Brochure (IB), and other relevant documents must be submitted to an Ethics Committee (EC) by the Investigator and reviewed and approved by the EC before the study is initiated.

Any substantial protocol amendments, likely to affect the safety of the subjects or the conduct of the study, will require EC and regulatory authority (as locally required) approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study subjects or any non-substantial changes, as defined by regulatory requirements.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the EC annually or more frequently in accordance with the requirements, policies, and procedures established by the EC.
- Notifying the EC of serious adverse events or other significant safety findings as required by EC procedures.
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 Code of Federal Regulations (CFR), ICH guidelines, the EC, European Directive 2001/20/EC for clinical studies (if applicable), and all other applicable local regulations.

Finances and Insurance

Financing and insurance will be addressed in a separate agreement.

Informed Consent

Prior to starting participation in the study, each subject will be provided with a study-specific ICF giving details of the study treatments, procedures, and potential risks of the study. Subjects will be instructed that they are free to obtain further information from the Investigator (or designee) and that their participation is voluntary and they are free to withdraw from the study at any time. Subjects will be given an opportunity to ask questions about the study prior to providing consent for participation.

Following discussion of the study with Clinical Research Unit (CRU) personnel, subjects will sign 2 copies of the ICF in the presence of a suitably trained member of staff to indicate that they are freely giving their informed consent. One copy will be given to the subject, and the other will be maintained in the subject's records.

Subjects must be re-consented to the most current version of the ICF(s) during their participation in the study.

Subject Data Protection

Subjects will be assigned a unique identifier and will not be identified by name in electronic Case Report Forms (eCRFs), study-related forms, study reports, or any related publications. Subject and Investigator personal data will be treated in compliance with all applicable laws and regulations. In the event the study protocol, study report, or study data are included in a public registry, all identifiable information from individual subjects or Investigator(s) will be redacted according to applicable laws and regulations.

The subject must be informed that his/her personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the subject. The subject must also be informed that his/her study-related data may be examined by Sponsor or Contract Research Organisation (CRO) auditors or other authorised personnel appointed by the Sponsor, by appropriate EC members, and by inspectors from regulatory authorities.

Disclosure

All information provided regarding the study, as well as all information collected and/or documented during the course of the study, will be regarded as confidential. The Investigator (or designee) agrees not to disclose such information in any way without prior written permission from the Sponsor.

The following data quality steps will be implemented:

- All relevant subject data relating to the study will be recorded on eCRFs unless directly transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by electronically signing the eCRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the eCRF.
- The Investigator must permit study-related monitoring, audits, EC review, and regulatory agency inspections and provide direct access to source data documents.
- Covance is responsible for the data management of this study including quality checking of the data. Predefined agreed risks, monitoring thresholds, quality tolerance thresholds, controls, and mitigation plans will be documented in a risk management register. Additional details of quality checking to be performed on the data may be included in a Data Management Plan.
- A Study Monitor will perform ongoing source data verification to confirm that data entered into the eCRF by authorised site personnel are accurate, complete,

and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

- Records and documents, including signed ICFs, pertaining to the conduct of this study must be retained by the Investigator in the study site archive for at least 5 years after the end of the study unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Investigator Documentation Responsibilities

All individual, subject-specific study data will also be entered into a 21 CFR Part 11-compliant electronic data capture (EDC) system on an eCRF in a timely fashion.

All data generated from external sources (eg, laboratory and bioanalytical data), and transmitted to Covance electronically, will be integrated with the subject's eCRF data in accordance with the Data Management Plan.

An eCRF must be completed for each enrolled subject who undergoes any screening procedures, according to the eCRF completion instructions. The Sponsor, or CRO, will review the supporting source documentation against the data entered into the eCRFs to verify the accuracy of the electronic data. The Investigator will ensure that corrections are made to the eCRFs and that data queries are resolved in a timely fashion by the study staff.

The Investigator will sign and date the eCRF via the EDC system's electronic signature procedure. These signatures will indicate that the Investigator reviewed and approved the data on the eCRF, data queries, and site notifications.

Publications

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

Appendix 6: Schedule of Assessments – Part A (Single-dose, Incorporating a Food-effect Evaluation)

Schedule of Assessments – Part A (Single Dose)

Study Procedures	Screening[l] (Days -28 to -2)	Treatment Period 1 (and 2 for Group A3)		Follow-up (7 to 10 days postdose)
		Day -1	Days 1 to 3	
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographic data	X			
Medical history	X	X[a]		
Urinary drug screen, including cotinine test	X	X		
Alcohol breath test	X	X		
Serology	X			
Pregnancy test[b]	X	X		X
FSH	X			
Height and body weight[c]	X	X		
[redacted]			[redacted]	
Study residency:				
Check-in		X		
Check-out			Day 3 (48 hours postdose)	
Non-residential visit	X			X
Study treatment administration:				
AM1476 or placebo			Day 1 (0 h) (30 minutes after starting a high-fat breakfast in Treatment Period 2 for Group A3)	
Pharmacokinetics:				
Blood sampling[e,f]			Predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36, and 48 hours postdose	
Urine sampling[e,f]			Predose (spot collection), 0 to 12, 12 to 24, and 24 to 48 hours postdose	
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X

Schedule of Assessments – Part A (Single Dose)

Study Procedures	Screening[l] (Days -28 to -2)	Treatment Period 1 (and 2 for Group A3)		Follow-up (7 to 10 days postdose)
		Day -1	Days 1 to 3	
Clinical chemistry, haematology, coagulation, and urinalysis	X	X	48 hours postdose	X
Blood pressure, pulse rate, respiratory rate, and oral body temperature	X		Predose and 1, 2, 4, 8, 24, and 48 hours postdose	X
Orthostatic vital signs	X		Predose and 1, 2, 4, 8, 24, and 48 hours postdose	X
12-lead ECG	X		Predose, and 1, 4, 8, 12, 24 and 48 hours postdose	X
Telemetry			From at least -1 hour predose to 24 hours postdose	
[redacted]			[redacted]	
Neurological examination	X		Days 1 (postdose) and 3[i]	X
Physical examination	X[j]	X[k]	Prior to discharge on Day 3[k]	X[k]

Abbreviations: CYP = cytochrome P450; ECG = electrocardiogram; FSH = follicle-stimulating hormone; PK = pharmacokinetic(s); PM = poor metaboliser(s); TMF = Trial Master File.

a. Interim medical history.
b. Performed in serum at Screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.
c. Height measured at Screening only.

[redacted]

f. Timepoints may be changed based on emerging data. Any changes to the scheduled times of PK assessments will be agreed with the Sponsor and documented in the TMF.

[redacted]

i. Symptom-directed neurological examination(s) will be performed on Day 1 and Day 3.
j. Full physical examination
k. Symptom-directed physical examination(s).
l. Group A1 screening period to be from Days -35 to -2.

Appendix 7: Schedule of Assessments – Part B (Multiple-dose)

Schedule of Assessments – Part B (Multiple Dose)

Study Procedures	Screening (Days -28 to -2)	Day -1	Days 1 to 12	Follow-up (7 to 10 days post-final dose)
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographic data	X			
Medical history	X	X[a]		
Urinary drug screen, including cotinine test	X	X		
Alcohol breath test	X	X		
Serology	X			
Pregnancy test[b]	X	X		X
FSH	X			
Height and body weight[c]	X	X		
[redacted]			[redacted]	
Study residency:				
Check-in		X		
Check-out			Day 12 (48 hours postdose)	
Non-residential visit	X			X
Study treatment administration:				
AM1476 or placebo			Day 1 to 10 (0 hour)	
Pharmacokinetics:				
Blood sampling[e,f]			Day 1: predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 hours postdose Days 4, 6, 7, 8, and 9: predose Day 10: predose, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, 36 and 48 hours postdose	
Urine sampling[e,f]			Day 1: predose (spot collection), 0 to 24 hours postdose Day 10: 0 to 24 hours postdose	

Schedule of Assessments – Part B (Multiple Dose)

Study Procedures	Screening (Days -28 to -2)	Day -1	Days 1 to 12	Follow-up (7 to 10 days post-final dose)
Safety and tolerability:				
Adverse event recording	X	X	Ongoing	X
Prior/concomitant medication monitoring	X	X	Ongoing	X
Clinical chemistry, haematology, coagulation, and urinalysis	X	X	Days 3 and 7: predose Day 10: 48 hours postdose	X
Blood pressure, pulse rate, respiratory rate, and oral body temperature	X		Day 1: predose, 2, 4, and 8 hours postdose Days 2, 4, and 7: predose Day 10: predose, 2, 4, 8, 24, and 48 hours postdose	X
Orthostatic vital signs	X		Day 1: predose, 2, 4, and 8 hours postdose Days 2, 4, and 7: predose Day 10: predose, 2, 4, 8, 24, and 48 hours postdose	X
12-lead ECG	X		Day 1: predose and 4 hours postdose Days 2, 4, and 7: predose and 4 hours postdose Day 10: predose, 4, 24, and 48 hours postdose	X
C-SSRS	X	X	Day 12	X
Neurological examination	X		Days 3, 6, 9, and 12[g]	X
Physical examination	X[h]	X[i]	Prior to discharge on Day 12[i]	X[i]

NOTE: the Schedule of Assessments for Part B is based on the dosing regimen being once daily for 10 days. However, the dietary state, fasting requirement, meal compositions, dosing duration, and dosing frequency may be changed following review of safety, tolerability, and PK data from Part A or earlier groups in Part B. The dose regimen will comprise no less than once every 2 days and will not exceed BID dosing. The dosing duration will comprise no fewer than 7 consecutive days and will not exceed 28 consecutive days. The number of assessments will not exceed those presented in the Schedule of Assessments though the timings may change based on ongoing review of safety, tolerability, and PK data.

Abbreviations: BID = twice daily; CSSRS = Columbia-Suicide Severity Rating Scale; CYP = cytochrome P450; ECG = electrocardiogram; FSH = follicle-stimulating hormone; PK = pharmacokinetic(s); PM = poor metaboliser(s); TMF = Trial Master File.

a. Interim medical history.
b. Performed in serum at Screening and in urine at all other times. A positive urine pregnancy test will be confirmed with a serum pregnancy test.
c. Height measured at Screening only.

[redacted]

[redacted]

f. Timepoints may be changed based on emerging data. Any changes to the scheduled times of PK assessments will be agreed with the Sponsor and documented in the TMF.
g. Symptom-directed neurological examination(s) will be performed on Day 3, Day 6, Day 9, and Day 12.
h. Full physical examination.
i. Symptom-directed physical examination(s).

Appendix 8: Summary of Amended Protocol Changes

The following changes were made:

[redacted]

2. A typographical error in the Schedule of Assessments for Part A was corrected.
3. An assessment of blood pressure, pulse rate, respiratory rate, and oral body temperature was added to the Schedule of Assessments at Follow-up for Part B.