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Avenida da Siderurgia Nacional
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Portugal

EudraCT N°: 2015-001799-24

Clinical Study Protocol N° BIA-102474-101

Biotrial Code: **1BIAL35**

A double-blind, randomised, placebo-controlled, combined single and multiple ascending dose study including food interaction, to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic profile of BIA 10-2474, in healthy volunteers

Investigational Medicinal Product Code:	BIA 10-2474
Development Phase:	I
Principal Investigator:	**Sponsor:**
Biotrial 7-9 rue Jean-Louis Bertrand, CS 34246 35042 Rennes Cedex France	BIAL – Portela & C^{a}, S.A. R&D Department Avenida da Siderurgia Nacional 4745-457 Coronado (S. Romão e S. Mamede) Portugal
Version 1.2	Date: 01 July 2015

Protocol Approval Form

The protocol entitled "*A double-blind, randomised, placebo-controlled, combined single and multiple ascending dose study including food interaction, to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic profile of BIA 10-2474, in healthy volunteers*", version 1.2 dated 01 July 2015 has been approved for submission to the French Ethics Committee and regulatory authorities (CPP and ANSM) by:

Sponsor's Representatives:

Head of Clinical Research 01.07.2015 **Date**

R&D Director , MD, PhD 02.07.2015 **Date**

Biotrial Medical Director:

03 JULY 2015 **Date**

Principal/ Coordinating Investigators' Approval

I, the undersigned, have examined this protocol and agree to conduct this trial according to this protocol, to comply with its requirements, subject to ethical and safety considerations, as set out in this protocol, the Declaration of Helsinki 1964 (latest revision Fortaleza 2013) and all other laws and regulations on the use of investigational medicinal products.

Date: 03 JUIL 2015.

Principal Investigator

1. SYNOPSIS

Name of Company: BIAL-Portela & C[a], S.A. À Avenida da Siderurgia Nacional, 4745-457 Coronado (S. Romão e S. Mamede) PORTUGAL
Name of Finished Product: NA
Name of active ingredient: BIA 10-2474
Title of Study: A double-blind, randomised, placebo-controlled, combined single and multiple ascending dose study including food interaction, to investigate the safety, tolerability, pharmacokinetic and pharmacodynamic profile of BIA 10-2474, in healthy volunteers
Principal Investigator:
Study centre: Biotrial, 7-9 rue Jean-Louis Bertrand, CS 34246, 35042 Rennes Cedex, France
Publication (reference): NA
Clinical Phase: Phase I
Rationale: This is the first study of single and multiple doses of BIA 10-2474 in human subjects. The current study is designed to assess the safety and tolerability of single and repeated oral doses of BIA 10-2474 in healthy subjects. The study also includes pharmacokinetic (PK)/pharmacodynamic (PD) characterization, to allow an estimate of the efficacious dose range to be studied in further clinical trials. The study further includes a preliminary assessment of the potential interaction of BIA 10-2474 with food in healthy young subjects. The tolerability, PK and PD data from this study will determine whether it is appropriate to continue development of BIA 10-2474.
Objectives: Primary: • To assess the safety and tolerability of BIA 10-2474 after single and multiple oral doses • To investigate the effect of food on the PK and PD of BIA 10-2474 Secondary: • To characterize the PK profile of BIA 10-2474 (and its metabolites) after single and multiple oral doses • To characterize its PD profile [mainly fatty acid amide hydrolase (FAAH) activity inhibition but also concentrations of N-arachidonoyl-ethanolamine (anandamide, AEA) and related fatty acid amides (FAAs) such as PEA (N-palmitoylethanolamide), OEA (N-oleoylethanolamide) and LEA (N-lineleoyl ethanolamide)] • To assess several potential PD effects
Design: A double blind, randomised, placebo-controlled combined single ascending dose (SAD) and multiple ascending dose (MAD) study, including an additional food interaction (FI) part (which is an open label design), and a PD part.

Design (continued):

The SAD part consists of 8 groups of 8 healthy young male and female subjects, gender balanced to the extent possible, each receiving a single oral dose of BIA 10-2474 or placebo (6 verum and 2 placebo). In the first group, 2 subjects (1 verum and 1 placebo) are to be dosed 24 h before the remaining 6 subjects, and if the safety and tolerability results are acceptable, the remaining 6 subjects (5 verum and 1 placebo) will be dosed. If the maximum tolerated dose (MTD) is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 12 sequential groups in total.

The FI part consists of 12 healthy young male and female subjects, gender balanced to the extent possible, each receiving either a single or multiple dose of BIA 10-2474 in either the fed or fasting state (to be decided after analysis of PK data available at this time) in an open-label, two-way crossover design. Treatment periods will be separated by at least 14 days (between the last administration of Period 1 and the first administration of Period 2).

The MAD part consists of 4 groups of 8 healthy young male and female subjects, gender balanced to the extent possible, each receiving an oral dose of BIA 10-2474 or placebo (6 verum and 2 placebo) once daily for 10 days. If the MTD is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 8 sequential groups in total.

The PD part consists of 1 group of 20 male subjects performing a double-blind, placebo-controlled, two-way cross-over design to assess PD effects of BIA 10-2474 on various PD models (pain, antitussive and anti-emetic), on intraocular pressure (IOP), on cognition and on mood. Treatment periods will be separated by at least 14 days (between the last administration of Period 1 and the first administration of Period 2). If necessary, the wash-out period can be extended according to the PK/PD data obtained in SAD/MAD parts.

Number of subjects:

One hundred and twenty-eight (128) healthy young subjects (64 in the SAD part, 12 in the FI part, 32 in the MAD part and 20 in the PD part). If the MTD is not reached after completing the planned SAD and MAD sequential groups, additional groups can be included in the SAD and MAD parts up to a maximum of 96 and 64 subjects, respectively.

Number of study centres:

1

Duration of participation:

Subjects will participate in the study for a maximum of 13 weeks.

Study products, dose and mode of administration:

Name	Formulation	Doses	Mode of administration
BIA 10-2474	Capsules, hard	0.25, 2.5 and 10 mg	Oral
Placebo	Capsules, hard	NA	Oral

SAD

Group S1: A single dose of 0.25 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S2: A single dose of 1.25 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S3: A single dose of 2.5 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S4: A single dose of 5 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S5: A single dose of 10 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S6: A single dose of 20 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S7: A single dose of 40 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S8: A single dose of 100 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1

Study products, dose and mode of administration (continued):

SAD (continued)

Escalation to the next higher dose and any dose adjustments of the next dose levels will be based on safety and tolerability results of the previously administered dose and available PK data of previous dose groups. There will be a 5-fold increase from the first dose level (0.25 mg) to the second dose level. The dose levels of the following groups will be increased by approximately 2-fold from the previous dose level, until the dose exceeds 100 mg, which is the human equivalent dose (HED) corresponding to the no observable adverse effect level (NOAEL) in the rat. Thereafter, there will be a 50% increase up to the last dose (if applicable). PK data of Group S1 through H72 after (last) dosing will be reviewed before the start of Group S2. Further PK data through H72 after (last) dosing [except if available PK data allow the review through H24 or H48 after (last) dosing] will be available with a lag time of 1 dose (i.e., PK results of Group S2 will be reviewed before the start of Group S4, etc.). The available PK results may change the planned dose levels.

If the MTD is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 12 groups.

FI

Group FI: The dose design of the food interaction will be decided during the SAD part (and the MAD part if available). The BIA 10-2474 dose and the use of single or multiple dose(s) will be decided based on previous PK data (nevertheless the daily dose will not exceed 33 % of the MTD or the maximum dose given in the SAD part). BIA 10-2474 will be administered in one period under fasting conditions and after a high-fat breakfast in the other period.

MAD

Groups M1 to M4: Multiple doses of BIA 10-2474 or matching placebo once daily from D1 to D10. The dose levels of Groups M1 to M4 will be determined after evaluation of the safety, tolerability and available PK results of previous SAD and MAD (when applicable) dose groups. The MAD part may start during the SAD part, but only when enough PK and safety data are available.

If the MTD is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 8 groups.

PD

Group PD: Repeated dosing from D1 to at least D10 with placebo and a well-tolerated BIA 10-2474 dose, chosen after analysis of previous SAD/MAD cohorts. These will be administered in a randomized order according to a cross-over design. The duration of each repeated dose is to be decided according to previous SAD and MAD PK results.

Product batches used will be identified in the Clinical Study Report (CSR).

Main eligibility criteria:

Inclusion:

Subjects must satisfy all of the following inclusion criteria before being allowed to participate/continue in the study:

1. Male or female subjects (only male subjects for the PD part) aged 18 to 55 years, inclusive;
2. Body mass index (BMI) between 19 and 30 kg/m^2 inclusive;
3. Healthy as determined by pre-study medical history, physical examination, vital signs, complete neurological examination and 12-lead electrocardiogram (ECG);
4. Negative tests for Hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV) and human immunodeficiency virus (HIV)-1 and HIV-2 antibody at screening;
5. Clinical laboratory test results clinically acceptable at screening and admission;
6. Negative screen for alcohol and drugs of abuse at screening and admission;
7. Non-smokers or ex-smokers (must have ceased smoking >3 months prior screening visit);

Main eligibility criteria (continued):

Inclusion (continued):

If female:

8. Woman with no childbearing potential by reason of surgery or at least 1 year post-menopause (i.e., 12 months post last menstrual period), or menopause confirmed by follicle-stimulating hormone (FSH) testing;
9. If of childbearing potential, using an effective nonhormonal method of contraception (intrauterine device or intrauterine system; condom or occlusive cap [diaphragm or cervical or vault caps] with spermicidal foam or gel or film or cream or suppository; true abstinence; or vasectomized male partner, provided that he is the sole partner of that subject) for all the duration of the study and up to one month after the last investigational medicinal product (IMP) administration;
10. Negative serum pregnancy test at screening and negative urine pregnancy test on admission of each treatment period (women of childbearing potential only);

If male:

11. Using an effective method of contraception (condom or occlusive cap [diaphragm or cervical or vault caps] with spermicidal foam or gel or film or cream or suppository; true abstinence; or vasectomy) throughout the study and up to one month after the last IMP administration.

For the PD part only:

12. Normal forced expiratory volume in one second (FEV1) at screening (80-120% predicted for the best FEV1 of 3 measures with a difference between the two best measures < or equal to 0.150 L);
13. Responsive (within acceptable ranges) to the cough provocation agent (capsaicin);
14. Normal basal intraocular pressure (IOP) (between 10-20 mmHg inclusive);
15. At screening, a cold pressor tolerance ≥15 seconds and ≤120 seconds on three measurements, after one training trial, with a deviation ≤20 seconds between the three measurements.

Exclusion:

If any of the following exclusion criteria apply, the subject must not enter/continue in the study:

1. Subjects who have a clinically relevant history or presence of respiratory, gastrointestinal, renal, hepatic, haematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine, connective tissue diseases or disorders;
2. Have a clinically relevant surgical history;
3. Have a history of hyperemesis;
4. Have a history of relevant atopy or drug hypersensitivity;
5. Have a history of alcoholism or drug abuse;
6. Consume more than 14 units of alcohol a week [1 glass (25 cl) of beer with 3° of alcohol = 7.5 g, or 1 glass (25 cl) of beer with 6° of alcohol = 15 g, or 1 glass (12.5 cl) of wine with 10° of alcohol = 12 g, or 1 glass (4cl) of aperitif with 42° of alcohol = 17 g];
7. Have a significant infection or known inflammatory process on screening or admission;
8. Have acute gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, heartburn) at the time of screening or admission;
9. Have used medicines within 2 weeks of admission that may affect the safety or other study assessments, in the investigator's opinion;
10. Have used any investigational drug or participated in any clinical trial within 90 days prior to screening;
11. Have participated in more than 2 clinical trials within the 12 months prior to screening;
12. Have donated or received any blood or blood products within the 3 months prior to screening;
13. Are vegetarians, vegans or have medical dietary restrictions;
14. Cannot communicate reliably with the investigator;
15. Are unlikely to co-operate with the requirements of the study;
16. Are unwilling or unable to give written informed consent.

Main eligibility criteria (continued):

Exclusion (continued):

If female:

17. Pregnancy or breast-feeding;
18. Woman of childbearing potential not using an accepted effective contraceptive method or using oral contraceptives;

If male:

19. Not using an accepted effective method of contraception.

Concomitant medications and study restrictions:

The use of medicines that, in the investigator's opinion, may affect the safety or other study assessments is prohibited within 2 weeks of admission.

The use of any investigational drug is prohibited within 90 days prior to screening.

Tobacco use is prohibited.

Subjects will be requested to abstain from strenuous physical activity, consumption of grapefruit or grapefruit-containing products, alcohol and stimulating beverages containing xanthine derivatives (i.e., no coffee, tea, chocolate or cola like drinks) for 48 hours prior to admission and until the follow up visit.

Procedures:

Screening:

Subjects will be screened for eligibility between D-28 and D-3 (D-2 for PD part) before the first study drug administration. Written informed consent will be obtained before any study procedure is performed. The screening will consist of: medical history; physical examination (including height and body weight); vital signs (supine and standing systolic and diastolic blood pressure (BP), pulse rate and tympanic body temperature); complete neurological examination; 12-lead ECG; haematology, coagulation, plasma biochemistry and urinalysis tests; HIV-1 and HIV-2, HBsAg and HCV serology; drugs of abuse and alcohol screen; and review of the eligibility criteria. Post-menopausal female subjects will be tested for FSH levels (if less than 12 months post last menstrual period) and female subjects of childbearing potential will be tested for pregnancy (serum test). For the subjects screened for the PD part, spirometry will be carried out to assess FEV1 and the basal IOP will be measured by an ophthalmologist using a contact tonometer. A cold pressor test and a cough challenge will also be performed in order to verify that the subjects are responsive (in predefined ranges) to pain and the cough provocation agent, respectively.

The results of screening must be known to the investigator prior to the subject's admission.

Admission:

Subjects will be admitted to the clinical unit one or two days prior to dosing (D-2 in the SAD, FI and MAD parts, D-1 in the PD part) to undergo medical history update; physical examination update; vital signs [supine and standing systolic BP (SBP) and diastolic BP (DBP), pulse rate (supine and standing SBP, DBP and pulse rate in triplicate on D-1) and tympanic body temperature]; 12-lead ECG (in triplicate on D-1); haematology, coagulation, plasma biochemistry (note that clinical laboratory assessments will be done on D-1 for all study parts), and urinalysis tests; drugs of abuse and alcohol screen; and verification of the eligibility criteria (at each study period if applicable). Female subjects of childbearing potential will be tested for pregnancy (urine test).

In the PD Part, psychometric tests and scales will be performed at t_{max} (if possible) on D-1. In SAD part and in PD part, familiarisation/training sessions will be done on D-1 for all psychometric tests and scales.

Procedures (continued):

Hospitalisation period:

- *SAD:* One (1) period in clinic from 36 h before drug administration to 72 h after drug administration on D1.
- *FI:* Two (2) periods in clinic (for each period from 36 h before drug administration to 72 h after drug administration) with a wash-out of at least 14 days (between the last administration of Period 1 and the first administration of Period 2).
- *MAD:* One (1) period in clinic from 36 h before the first drug administration to 72 h after the last drug administration on D10.
- *PD:* 2 periods in clinic from 24 h before the first drug administration to 48 h after the last drug administration (at least D10), with a wash-out of at least 14 days (between the last administration of Period 1 and the first administration of Period 2).

Follow-up:

A follow-up (FU) visit will occur between 14 and 21 days after discharge or early discontinuation for: medical history and physical examination updates; vital signs (supine and standing systolic and diastolic blood pressure, pulse rate and tympanic body temperature); 12-lead ECG; haematology, coagulation, plasma biochemistry and urinalysis tests. Female subjects of childbearing potential will be tested for pregnancy (serum test).

Criteria for evaluation:

Safety Assessments while on treatment:

- *SAD:* Adverse events (AEs): throughout the study; physical examination: throughout the study on medical indication at the discretion of the Medical Investigator; Supine and standing vital signs: on D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; tympanic body temperature: at D4/discharge; ECG on D-1 (in triplicate), on D1 at pre-dose, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; clinical laboratory evaluation: at discharge; telemetry: from pre-dose to 24 h post-dose.

- *FI:* AEs: throughout the study; physical examination: throughout the study on medical indication at the discretion of the Medical Investigator; Supine and standing vital signs: on D-1 (in triplicate), on D1 at pre-dose, 1, 2, 3, 4, 8, 12, 24, 48 and 72 h post-single or last dose; tympanic body temperature: at D4/discharge; ECG on D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 24 and 72 h post-single or last dose; clinical laboratory evaluation: at discharge.

- *MAD:* AEs: throughout the study; physical examination: throughout the study on medical indication at the discretion of the Medical Investigator; Supine and standing vital signs: on D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose; on D10 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; tympanic body temperature: on D13/discharge; ECG on D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose and on D10 at pre-dose, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; clinical laboratory evaluation: on D5 and at discharge; telemetry: from pre-dose to 24 h post-dose on D1 and D10.

- *PD*: AEs: throughout the study; physical examination: throughout the study on medical indication at the discretion of the Medical Investigator; Supine and standing vital signs: On D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post dose; on D9 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; tympanic body temperature: on D12/discharge. ECG on D-1 (in triplicate), on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post dose and on D9 at pre-dose, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; clinical laboratory evaluation: at discharge.

Criteria for evaluation (continued):

Pharmacokinetic Assessments in blood

- *SAD*: Blood sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose.

Maximum observed plasma concentration (C_{max}), time of occurrence of C_{max} (t_{max}), apparent terminal elimination rate constant (λ_z), terminal half-life ($t_{½}$), area under the plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}), area under plasma concentration-time curve from hour 0 to infinity (AUC_{inf}), apparent volume of distribution (V_z/F), total body clearance (CL/F), time to last measurable plasma concentration (t_{last}), last measurable plasma concentration (C_{last}); additional parameters could be calculated if deemed necessary.

- *FI*: Blood sampling for PK of BIA 10-2474 [and/or metabolites, depending on the previous SAD PK data and decided in accordance with bioavailability/bioequivalence (BABE) guidelines]: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-single or last dose.

C_{max}, t_{max}, λ_z, $t_{½}$, AUC_{last}, AUC_{inf}, V_z/F, CL/F, t_{last}, C_{last}; additional parameters could be calculated if deemed necessary.

- *MAD*: Blood sampling for PK of BIA 10-2474 and metabolites: pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose on D1; pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 60 and 72 h post-dose on D10; pre-dose on D4, D6 and D8.

D1: C_{max}, C_{last}, t_{max}, t_{last}, AUC_{last}, area under the plasma concentration-time curve over one dosing interval ($AUC_{0-\tau}$);

D10: C_{max}, C_{last}, t_{max}, t_{last}, $AUC_{0-\tau}$, , λ_z, $t_{½}$, V_z/F, CL/F, AUC_{inf}, AUC_{last}, minimum observed plasma concentration (C_{min}), average plasma concentration at steady state ($C_{average}$), accumulation ratio of C_{max} ($R_{acc}C_{max}$), accumulation ratio of $AUC_{0-\tau}$ (R_{acc} $AUC_{0-\tau}$), concentration at the end of a dosing interval before the next dose administration (C_{trough}), peak-trough fluctuation (PTF%); additional parameters could be calculated if deemed necessary.

Pharmacokinetic Assessments in Urine

- *SAD*: Urine sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 h post-dose intervals.

Total amount excreted in urine (Ae), percent of drug recovered in urine (Ae %dose) and renal clearance (CL_r); additional parameters could be calculated if deemed necessary.

- *MAD*: Urine sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0-4, 4-8, 8-12 and 12-24 h post-dose intervals on D1; pre-dose and 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 h post-dose intervals on D10.

Ae, Ae %dose and CL_r; additional parameters could be calculated if deemed necessary.

Pharmacodynamic Assessments

- *SAD:* Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16, 12 h before dosing on D1 (corresponding to the time points on D1) and pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose.

Blood sampling for analysis of AEA and related FAAs: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose.

- *FI:* Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16 and 12 h before dosing on D1 (corresponding to the time points on D1); pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-single or last dose.

Pharmacodynamic Assessments (continued)

- *MAD:* Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16 and 12 h before dosing on D1 (corresponding to the time points on D1); pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose on D1; pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72h post-dose on D10; pre-dose on D4, D6 and D8.

Blood sampling for analysis of AEA and related FAAs: pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose on D1; pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 60 and 72 h post-dose on D10; pre-dose on D4, D6 and D8.

- *SAD, MAD and FI parts:* Maximum observed effect on FAAH activity (E_{max}), time to occurrence of E_{max} (t_{Emax}), area under the effect-time curve (AUEC).

Anandamide (AEA) and related FAAs like PEA (N-palmitoylethanolamide), OEA (N-oleoylethanolamide) and LEA (N-lineleoyl ethanolamide) will also be analysed.

- *Psychometric tests and scales*
- *SAD*: Marijuana scale score assessed on D-1 (familiarisation); pre-dose and 3 h post-dose on D1.

- *PD*: Number of correct answers in Choice Reaction Time (CRT) and Digit Vigilance (DV), number of correctly detected targets in Rapid Information Processing Test (RVIP), mean response time in CRT, DV and RVIP, number of missed targets and number of false answers for DV and RVIP, number of words correctly recalled in immediate recall 1, mean number of words correctly recalled in all immediate recalls and number of words recalled in delayed recall in Learning Memory Test (LMT), sleepiness score for Stanford Sleepiness Scale (SSS) and tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment scores in Profile of Mood States (POMS). These tests will be performed on D-1 (familiarisation); at t_{max} on D1, D5 and D9 (POMS, DV, CRT, RVIP and SSS) and at t_{max} (if possible) on D-1 and D9 (LMT).

- *Cold pressor test*
- *PD:* Time endured with hand submerged in cold water (cold pressor tolerance-CP_{tol}), maximum value observed (Pain VAS_{max}), total of values observed until the withdrawal from the bath (Pain VAS_{total}), analogue scale with replacement of missing values between time of withdrawal and 120 seconds by the maximum value (100) (Pain $AUC_{0\text{-}120s}$). This test will be performed at screening and at t_{max} on D6.
- *Cough challenge*
- *PD:* C2, C5, number of coughs during the first 15s after administration for each capsaicin concentration, number of coughs from 15s after to 1 minute after administration for each capsaicin concentration.This test will be performed at screening and at t_{max} on D7.

- *IOP measurement*
- *PD:* IOP in mmHg. This test will be performed at screening and at t_{max} on D8.

- *Apomorphine challenge*

PD: Maximum degree of nausea (NS_{max}), time of the maximum degree of nausea (NS t_{max}), area under the degree of nausea versus time curve (NS $AUC_{0\text{-}90min}$). This test will be performed at t_{max} on D10.

Statistical methods:

Data entry and statistical analysis of clinical parameters will be performed under the responsibility of Biotrial.

The statistical package SAS® (SAS Institute Inc. Cary NC USA) will be used in statistical analysis.

Safety parameters:

All laboratory results, vital signs measurements, and safety ECG results will be summarised using appropriate descriptive statistics. The incidence of all AEs and treatment-emergent AEs will be described by MedDRA® preferred term and system organ class.

Statistical methods (continued):

PK parameters:

Single and multiple-dose PK parameters will be derived from the plasma concentration time and urinary excretion data.
A compartmental or non-compartmental PK method, as appropriate, will be used to analyse the plasma and urine concentrations of BIA 10-2474 and its metabolites.
Descriptive statistics and graphs will be performed for plasma and urine concentrations and PK parameters.
For the SAD and MAD parts, the dose-proportionality of PK plasma parameters C_{max} and AUC will be evaluated using the Power model.
For the MAD part, the steady-state of plasma concentration will be also studied using a one-way analysis of variance (ANOVA) on factor Day after logarithmic transformation of plasma concentrations.
For the FI part, an analysis of variance model appropriate for a 2-period, cross-over design with fixed terms for sequence, period, and treatment and a random term for subject within sequence will be used to investigate the food interaction. Following log-transformation of the data, the 90% confidence intervals (90%CI) for the geometric mean ratio (GMR) between the food conditions will be calculated. Moreover, t_{max} will be compared between the food conditions using a Wilcoxon signed ranks test.

PD parameters:

All parameters will be summarised using appropriate descriptive statistics. The differences between treatment group and placebo group will be investigated using appropriate statistical tests.

Flow chart 1: SAD part

PROCEDURES	Screening (D-28 to D-3)	Admission (D-2 evening)	D-1	Day 1	Day 2	Day 3	Day 4/Discharge	Follow-up (between D18 & D25)
Written informed consent	X							
Medical history	X							
Medical history update		X						X
Physical examination[1]	X							
Physical exam update		X						X
Vital signs[2]	X	X	X	X	X	X	X	X
Complete neurological examination	X							
12-lead ECG[3]	X	X	X	X	X	X	X	X
Viral serology	X							
Alcohol and drugs of abuse screen	X	X						
Haematology	X		X				X	X
Coagulation	X		X				X	X
Plasma biochemistry	X		X				X	X
Urinalysis	X	X					X	X
Serum pregnancy test[4]	X							X
Hormone Panel (FSH testing)[5]	X							
Urine pregnancy test[4]		X						
Eligibility criteria review	X	X		X[6]				
Randomisation				X				
Pharmacokinetic sampling: blood[7]				X	X	X	X	
Pharmacokinetic sampling: urine[8]				X	X	X	X	
Pharmacodynamic sampling: blood[9]			X	X	X	X	X	
Marijuana scale score[10]			X	X				
Telemetry[11]				X	X			
Study drug administration				X				
Adverse events monitoring	←							→

[1] Physical examination at screening; from D-2 to D4 on medical indication at the discretion of the Medical Investigator.
[2] Blood pressure, pulse rate, body temperature. *Supine and standing vital signs*: at screening, D-2 (admission), D-1 (in triplicate), and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; *Body temperature* will be measured at screening, D-2, D4 (discharge) and follow-up visit only.
[3] ECG at screening, D-2, D-1 (in triplicate), and at follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose.
[4] Female subjects of childbearing potential only.
[5] Post-menopausal female subjects only if less than12 months post last menstrual period.
[6] Before randomization.
[7] Blood sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose. Blood sampling for analysis of AEA and related FAAs: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose.
[8] Urine sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 h post-dose intervals.
[9] Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16, 12 h before dosing on D1 (same time points as on D1) and pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose
[10] Psychometric scale: M Scale (marijuana) at D-1 (familiarization), at predose and at 3 h post-dose.
[11] Telemetry: from pre-dose to 24 h post-dose.

Flow chart 2: FI part

		Assessment Period 1 and Period 2 (wash-out: 14 days)						
PROCEDURES	Screening (D-28 to D-3)	Admission (D-2 evening)	D-1	Day 1	Day 2	Day 3	Day 4/Discharge	Follow-up (between D18 & D25)
Written informed consent	X							
Medical history	X							
Medical history update		X						X
Physical examination[1]	X							
Physical exam update		X						X
Vital signs[2]	X	X	X	X	X		X	X
Complete neurological examination	X							
12-lead ECG[3]	X	X	X	X	X		X	X
Viral serology	X							
Alcohol and drugs of abuse screen	X	X						
Haematology	X		X				X	X
Coagulation	X		X				X	X
Plasma biochemistry	X		X				X	X
Urinalysis	X	X					X	X
Serum pregnancy test[4]	X							X
Hormone Panel (FSH testing)[5]	X							
Urine pregnancy test[4]		X						
Eligibility criteria review	X	X		X_6				
Randomisation				X				
Pharmacokinetic sampling: blood[7]				X	X	X	X	
Pharmacodynamic sampling: blood[8]			X	X	X	X	X	
Study drug administration[9]				X				
Adverse events monitoring	◄							►

[1] Physical examination at screening; from D-2 to D4 on medical indication at the discretion of the Medical Investigator.
[2] Blood pressure, pulse rate, body temperature. *Supine and standing vital signs*: at screening, D-2 (admission), D-1 (in triplicate), and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 8, 12, 24, 48 and 72 h post-single or last dose; *Body temperature* will be measured at screening, D-2, D4 and follow-up visit only.
[3] ECG at screening, D-2, D-1 (in triplicate), and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 24 and 72 h post-single or last dose.
[4] Female subjects of childbearing potential only.
[5] Post-menopausal female subjects only if less than12 months post last menstrual period.
[6] Before randomization.
[7] Blood sampling for PK of BIA 10-2474 and metabolites: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-single or last dose.
[8] Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16 and 12 h before dosing on D1 (same time points as on D1); pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-single or last dose.
[9] Study drug will be administered after an overnight fast in one period and after consumption of a high fat breakfast in the other period.

Flow chart 3: MAD part

PROCEDURES	Screening (D-28 to D-3)	Admission (Day -2 evening)	D-1	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13 /Discharge	Follow up (between D27 &D34)
Written informed consent	X																
Medical history	X																
Medical history update		X															X
Physical examination[1]	X																
Physical exam update		X															X
Vital signs[2]	X	X	X	X	X								X	X	X	X	X
Complete neurological examination	X																
12-lead ECG[3]	X	X	X	X	X								X	X	X	X	X
Viral serology	X																
Alcohol and drugs of abuse screen	X	X															
Haematology	X		X					X[4]								X	X
Coagulation	X		X					X[4]								X	X
Plasma biochemistry	X		X					X[4]								X	X
Urinalysis	X	X						X[4]								X	X
Serum pregnancy test[5]	X																X
Hormone Panel (FSH testing)[6]	X																
Urine pregnancy test[5]		X															
Eligibility criteria review	X	X		X[7]													
Randomisation				X													
Pharmacokinetic sampling: blood[8]				X	X		X		X		X		X	X	X	X	
Pharmacokinetic sampling: urine[9]				X	X								X	X	X	X	
Pharmacodynamic sampling: blood[10]			X	X	X		X		X		X		X	X	X	X	
Telemetry[11]				X	X								X	X			
Study drug administration				X	X	X	X	X	X	X	X	X	X				
AE monitoring	◄																►

[1] Physical examination at screening; from D-2 (admission), to D13 (discharge) on medical indication at the discretion of the Medical Investigator.

[2] Blood pressure, pulse rate, body temperature. *Supine and standing vital signs*: at screening, D-2 (admission), D-1 (in triplicate), and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose; on D10 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose; *Body temperature* will be measured at screening, D-2, D13 and follow-up visit only.

[3] ECG at screening, D-2, D-1 (in triplicate), and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose and on D10 at pre-dose, 1, 2, 3, 4, 6, 8, 10, 12, 24, 48 and 72 h post-dose.

[4] Clinical laboratory: on D5 it will be performed at pre-dose.

[5] Female subjects of childbearing potential only.

[6] Post-menopausal female subjects only if less than12 months post last menstrual period.

[7] Before randomization.

[8] Blood sampling for PK of BIA 10-2474 and metabolites: pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose on D1; pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48, 60 and 72 h post-dose on D10; pre-dose on D4, D6 and D8.
Blood sampling for analysis of AEA and related FAAs: pre-dose and 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose.

[9] Urine sampling for PK of BIA 10-2474 and metabolites: pre-dose, 0-4, 4-8, 8-12 and 12-24 h post-dose intervals on D1; pre-dose, 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-60 and 60-72 h post-dose intervals on D10.

[10] Blood sampling for FAAH activity: 24, 23.5, 23, 22, 21, 20, 18, 16 and 12 h before dosing on D1 (same time points as on D1); pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 h post-dose on D1; pre-dose, 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 and 72 h post-dose on D10; pre-dose on D4, D6 and D8.

[11] Telemetry: from pre-dose to 24 h post-dose on D1 and D10.

Flow chart 4: PD part

		Assessment Period 1 and Period 2 (wash–out: 14 days)													
PROCEDURES	Screening (D-28 to D-2)	Admission (Day -1)	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12 Discharge	Follow-up (between D26 & D33)
Written informed consent	X														
Medical history	X														
Medical history update		X													X
Physical examination[1]	X													X	
Physical exam update		X													X
Vital signs[2]	X	X	X	X							X	X	X	X	X
Complete neurological examination	X													X	
12-lead ECG[3]	X	X	X	X							X	X	X	X	X
Viral serology	X														
Alcohol and drugs of abuse screen	X	X													
Haematology	X	X												X	X
Coagulation	X	X												X	X
Plasma biochemistry	X	X												X	X
Urinalysis	X	X												X	X
Spirometry	X														
Eligibility criteria review	X	X	X[4]												
Cold pressor test	X							X							
Cough challenge	X								X						
IOP measurement	X									X					
Randomisation			X												
Psychometric tests[5]		X	X				X				X				
Apomorphine challenge												X			
Study drug administration			X	X	X	X	X	X	X	X	X	X			
Adverse events monitoring	◄														►

[1] Physical examination at screening and follow-up; from D-1 (admission), to D12 (discharge) on medical indication at the discretion of the Medical Investigator.

[2] Blood pressure, pulse rate, body temperature. *Supine and standing vital signs*: at screening, D-1 (admission) (in triplicate) and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose; on D9 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12, 24 h and 48 post-dose, and on D12 before discharge; *Body temperature* will be measured at screening, D-1, D12 and follow-up visit only.

[3] ECG at screening, D-1 (in triplicate) and follow-up; on D1 at pre-dose and 1, 2, 3, 4, 6, 8, 10, 12 and 24 h post-dose and on D9 at pre-dose, 1, 2, 3, 4, 6, 8, 10, 12, 24 and 48 h post-dose, and on D12 before discharge.

[4] Before randomization.

[5] Psychometric tests and scales will be performed at t_{max} on D1, D5 and D9 (POMS, DV, CRT, RVIP and SSS) and at t_{max} (if possible) on D-1 and D9 (LMT). Familiarisation/training sessions will be done on D-1 for all psychometric tests and scales.

TABLE OF CONTENTS

List of Tables

LIST OF ABBREVIATIONS

AE	Adverse Event
A_e	Total amount excreted in urine
$A_{e\%dose}$	Percent of drug recovered in urine
AEA	Anandamide or N-arachinoylethanolamine
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
ANSM	Agence Nationale de Sécurité du Médicament et des produits de santé
APPT	Activated Partial Thromboplastin Time
ARCI	Addiction Research Center Inventory
AST	Aspartate Amino Transferase
AUC	Area Under the plasma concentration-time Curve
AUC_{0-t}	Area under the plasma concentration-time curve from time 0 to the time of last quantifiable concentration
AUC_{0-120}	Pain Analogue scale between time of withdrawal and 120s by maximum value
AUEC	Area Under the Effect-time Curve
AUC_{inf}	Area Under the plasma concentration-time Curve from hour 0 to infinity
AUC_{last}	Area Under the plasma concentration-time Curve from hour 0 to last sample with measurable plasma concentrations
BABE	Bioavailability/Bioequivalence
BMI	Body Mass Index
BP	Blood Pressure
CI	Confidence Interval
cm	centimetre
$C_{average}$	Average plasma concentration at steady state
C_{max}	Maximum observed plasma concentration
C_{min}	Minimum observed plasma concentration
C_{last}	Last measurable plasma concentration
CL_r	Apparent Renal Clearance
CL/F	Apparent total body Clearance
CPK	Creatinine Phosphokinase
CPP	Comité de Protection des Personnes (Independent Ethics Committee)
CP_{tol}	Cold pressor tolerance
CRO	Contract Research Organisation
CRF	Case Report Form
CRT	Choice Reaction Time

CSR	Clinical Study Report
C_{trough}	Concentration at the end of a dosing interval before the next dose administration
CTD	Common Technical Document
CV	Coefficient of Variation
C2	Concentrations of capsaicin causing 2 coughs
C5	Concentrations of capsaicin causing 5 coughs
D	Day
DBP	Diastolic Blood Pressure
DMSO	Dimethylsulphoxide
DV	Digit Vigilance
EA-d4	Deuterated ethanolamide
E_{max}	Maximum observed effect on FAAH activity
ECG	Electrocardiogram
ERS	European Respiratory Society
EudraCT	European Clinical Trials Database
FAAH	Fatty Acid Amide Hydrolase
FEV1	Forced Expiratory Volume in 1 second
FI	Food Interaction
FSH	Follicle-stimulating hormone
FU	Follow up
GGT	Gamma Glutamyl Transferase
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GMR	Geometric mean ratio
Hb	Haemoglobin
HBsAg	Hepatitis B surface Antigen
HCV	Hepatitis C Virus
HDPE	High-Density PolyEthylene
HED	Human Equivalent Dose
HIV	Human Immunodeficiency Virus
HR	Heart Rate
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier

IND	Investigational New Drug application
IRB	Institutional Review Board
INR	International Normalized Ratio
IOP	Intra ocular Pressure
kg	kilogram
λ_z	Apparent terminal elimination rate constant
LEA	N-lineleoyl ethanolamide
LDH	Lactate Dehydrogenase
LMT	Learning Memory Test
min	minute
mL	millilitre
mmHg	Millimetre of mercury
msec	millisecond
M	Marijuana
MAD	Multiple Ascending Dose
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum Tolerated Dose
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NS	Nausea
NS_{max}	Maximum Degree of Nausea
NS_{tmax}	Time of maximum degree of nausea
NS $AUC_{0-90min}$	Area under the degree of nausea versus time curve
OEA	N-oleoylethanolamide
PEA	N-palmitoylethanolamide
PBS	Phosphate-buffered saline
PD	Pharmacodynamic
PK	Pharmacokinetics
pmol	picomole
p. o.	*per os*
POMS	Profile of Mood States
PR	Pulse Rate
QRS	QRS complex duration (ECG)
QT	QT interval (ECG)
QTc	QT interval corrected for heart rate
QTcB	QT interval corrected for heart rate using Bazett's correction
QTcF	QT interval corrected for heart rate using Fridericia's correction
R_{ac}	Accumulation ratio of C_{max}
RVIP	Rapid Vigilance Information Processing

SAE	Serious Adverse Event
SAD	Single Ascending Dose
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SEM	Standard Error of Mean
SOP	Standard Operating Procedure
SSS	Stanford Sleepiness Scale
SUSARs	Suspected Unexpected Serious Adverse Reactions
τ	Dosing interval
$t_{1/2}$	Apparent terminal elimination half-life
t_{Emax}	Time to occurrence of E_{max}
t_{last}	Time to last measurable plasma concentration
t_{max}	Time to reach C_{max} (time at peak plasma level)
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
VAS	Visual Analogue Scale
VAS_{max}	Maximum value observed
VAS_{total}	Total of values observed until the withdrawal from the bath
V_z/F	Apparent volume of distribution
WHO-DD	World Health Organisation Drug Dictionary
2-AG	2-arachidonoylglycerol
%	percent

2. BACKGROUND AND RATIONALE

2.1. Background

2.1.1. The endocannabinoid system

The endocannabinoid system has been implicated in a growing number of physiological functions, both in the central and peripheral nervous systems, and in peripheral organs. More importantly, modulating the activity of the endocannabinoid system has turned out to hold therapeutic promise in a wide range of disparate diseases and pathological conditions, ranging from mood and anxiety disorders, movement disorders such as Parkinson's and Huntington's diseases, neuropathic pain, multiple sclerosis and spinal cord injury, to cancer, atherosclerosis, myocardial infarction, stroke, hypertension, glaucoma, obesity/metabolic syndrome, and osteoporosis. The endogenous analogues of cannabinoids, the "endocannabinoids", include anandamide (N-arachidonoylethanolamine, AEA), 2-arachidonoylglycerol (2-AG), and other amides, esters and ethers of long chain polyunsaturated fatty acids. Fatty acid amide hydrolase (FAAH), the enzyme responsible for the degradation of AEA *in vivo*, has emerged as a promising target for modulating endocannabinoid signalling, with therapeutic potential in several medical conditions.

2.1.2. BIA 10-2474

BIA 10-2474 (3-(1-(cyclohexyl(methyl)carbamoyl)-1H-imidazol-4-yl)pyridine 1-oxide) is currently being developed by Bial - Portela & C[a], S.A. (BIAL) for the treatment of medical conditions in which there is an advantage in enhancing the levels of endogenous AEA and tonically increasing the drive of the endocannabinoid system. BIA 10-2474 is a reversible FAAH inhibitor that increases AEA levels in the central nervous system and in peripheral tissues. It is designed to act as a long-acting and reversible inhibitor of brain and peripheral FAAH, endowed with sustained inhibition of the enzyme.

2.1.2.1. Non-clinical data

BIA 10-2474 inhibited FAAH activity in mouse brain and liver in a time- and dose-dependent manner. Decreases in FAAH activity after BIA 10-2474 administration were accompanied by increases in brain AEA levels, in a time- and dose-dependent manner. BIA 10-2474 was tested in models of predictive efficacy in pain conditions. When given alone BIA 10-2474 produced analgesic/anti-inflammatory activity in the mouse Formalin-Paw and Tail-Flick tests in a time-and dose-dependent manner. BIA 10-2474 also markedly potentiated the antinociceptive effects of exogenous AEA in the mouse Formalin-Paw and Tail-Flick tests.

Safety pharmacology evaluation revealed that BIA 10-2474 up to 300 mg/kg (p.o.) had no significant effects on the gross behavioural state, gastrointestinal transit and renal function in the rat. BIA 10-2474 was found not to affect HERG mediated currents up to 10 μM, which corresponds to 3 μg/mL. BIA 10-2474 (1.5, 4.5 and 15 μg/mL) had no effects on the action potential parameters in the dog Purkinje fiber. In dogs, BIA 10-2474 had no major effects on arterial blood pressure (BP), heart rate, and on the PR, the QRS, the QT and the QTc (Fridericia's and van de Water's formulae) intervals. No arrhythmia or other changes in the morphology of the electrocardiogram which could be attributed to BIA 10-2474 were observed.

Animal toxicology studies of repeated daily dosing of BIA 10-2474 for up to 13 weeks in mice, dogs and monkeys and up to 26 weeks in rats have been conducted. Treatment with BIA 10-2474 produced no signs of toxicity in mice, rats, dogs and monkeys up to the no observed adverse effect level (NOAEL) indicated in the table below. Administration of BIA 10-2474 was associated with dose-dependent increases in plasma level. The table below indicates the extent of exposure (AUC_{0-t} [ng•h/mL]) of BIA 10-2474 associated with the corresponding NOAEL at the end of treatment.

Study	Study code	NOAEL (mg/kg/day)	AUC_{0-t} (ng•h/mL)	
			Males	Females
Four week oral repeat administration in the mouse	C74568	100	160,000	149,000
Three month oral repeat administration in the mouse	C75042	25	30,700	24,900
Four week oral repeat administration in the rat	C74570	30	41,300	64,900
Three month oral repeat administration in the rat	C75031	10	18,900	23,100
Six month oral repeat administration in the rat	D35972	10	16,751	20,109
Four week oral repeat administration in the dog	S30940	50	65,000	38,600
Three month oral repeat administration in the dog	S31208	20	24,100	66,200
Four week oral repeat administration in the monkey	S38498	100	179,416	168,772
Three month oral repeat administration in the monkey	S47972	75	138,325	130,702

The genotoxic profile of BIA 10-2474 was examined in two types of tests. BIA 10-2474 was not mutagenic in the Ames test, when tested in dimethylsulphoxide (DMSO) up to the limit of its solubility, suggesting that there is no indication of a genotoxic risk to humans. The *in vivo* genotoxic potential of BIA 10-2474 was evaluated for its potential to cause chromosome or cell division apparatus damage, or cell cycle interference, leading to micronucleus formation in polychromatic erythrocytes in the bone marrow of mice; it was concluded that BIA 10-2474 did not induce micronuclei in bone marrow cells when tested to the maximum tolerated dose of 1800 mg/kg/day in male CD-1 mice and 1400 mg/kg/day in female CD-1 mice using a 0 h and 24 h oral dosing and 48 h sampling regimen.

Reproductive and development toxicology studies were performed in rats and rabbits and the no effect level (NOEL) for each particular study segment is indicated in the table below.

Study	Code	NOEL (mg/kg/day)
Rat fertility and early embryonic development (Segment I)	C75086	50
Rat developmental toxicity study (Segment II)	C75108	Maternal = 25 Fetal = 7.5
Pre- and post-natal developmental toxicity study in the rat (Segment III)	C75110	F1 generation = 6
Rabbit study for effects on embryo-fetal development (Segment II main test)		Maternal = 25 Fetal = 25

In human hepatic microsomes, BIA 10-2474 (up to 30 µg/mL) produced minor inhibition in CYP2D6 and CYP3A4 (testosterone) and is not an inhibitor of CY1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2E1 and CYP3A4 (midazolam) activities *in vitro*. BIA 10-2474 (up to 30 µg/mL) is not considered to be an inducer of CYP2B and CYP3A, although further investigation with CYP1A2 may be warranted due to the effect observed in one of the donors used in this study. It has been predicted that the maximum plasma concentration of BIA 10-2474 achieved in humans is not likely to be >2 µg/mL and, therefore, it is unlikely that these inhibitions will be of clinical significance.

2.1.2.2. Pharmacokinetics and metabolism

Administration of BIA 10-2474 was associated with dose-dependent increases in plasma level. In both rats and dogs, [^{14}C]-BIA 10-2474 was detectable up to 24 h post-dose. The apparent terminal half-life ($t_{1/2}$) of total radioactivity was 45 h and 104 h and 4 h and 52 h following oral and intravenous administration, respectively. In both rats and dogs, the urinary excretion was the primary route of excretion of total radioactivity, accounting for 65.2 and 68.7 %, and 50.8 and 61.2% of the administered dose, following both oral and intravenous administration of [^{14}C]-BIA 10-2474. Faecal excretion was the secondary route of excretion, accounting for 23.5 and 18.3%, and 23.8 and 22.2% of the administered dose, following both oral and intravenous administration of [^{14}C]-BIA 10-2474. Excretion of total radioactivity following both oral and intravenous administration of [^{14}C]-BIA 10-2474 occurred principally in the first 24 to 48 h after dose administration and was essentially complete by 72 h post-dose. In the mouse, rat, dog and monkey, BIA 10-2474 undergoes extensive metabolism.

2.1.2.3. Effects in humans

Up to the date of this document, there is no experience with BIA 10-2474 in human subjects.

Subjects should be instructed to take BIA 10-2474 only as recommended. Since BIA 10-2474 increases exposure to anandamide, endocannabinoid effects, such as catalepsy, hypothermia and hyperphagia, may be potentiated. Subjects should be informed about the possible occurrence of such effects and cautioned against rising rapidly after sitting or lying down. Subjects should be advised not to drive or operate dangerous machinery. Because of potential additive sedative effects, caution should be used when taking other CNS depressants.

For more detailed information, please refer to the Investigator's Brochure (IB) [1].

2.1.3. Study rationale

2.1.3.1. Regulatory background

This is the first study of single and multiple doses of BIA 10-2474 in human subjects. The current study is designed to assess the safety and tolerability of single and repeated oral doses of BIA 10-2474 in healthy subjects. The study also includes pharmacokinetic (PK)/pharmacodynamic (PD) modelling, to allow an estimate of the efficacious dose range to be studied in further clinical trials. The assessment of the potential interaction of BIA 10-2474 with food in healthy young subjects is also to be investigated.

The tolerability, PK and PD data from this study will determine whether it is appropriate to continue development of BIA 10-2474.

2.1.3.2. Subject population

As is usual in first-in-man studies, the population to be included is healthy young males and females between 18 and 55 years old (inclusive) at screening. The selection of healthy subjects is justified on the basis that PK, safety, and tolerability, can be investigated accurately in this population.

In healthy subjects, the study can be performed under standardized conditions, reducing the influence of confounding factors.

According to international guidelines, as soon as the pre-clinical studies allow the inclusion of women, female subjects should be included in Phase I studies in order to appreciate safety, PK and PD in each gender. There are known sex differences in how women and men absorb, metabolize and excrete certain therapeutic products (for example some antidepressants, antipsychotics or antibiotics). It is theorized that these pharmacokinetic differences stem from variations between the sexes due to factors such as body weight, plasma volume, gastric emptying time, plasma protein levels, metabolizing enzymes, drug transporter function and clearance activity. Sex differences in pharmacodynamics have been observed in drugs acting on the central nervous system, immune system, cardiovascular system and on energy metabolism.

For this reason, it has been decided to include female subjects in this Phase I study nevertheless, in the PD part, as the objectives of this specific part are very specific, the choice to include only men was done considering the gender effect in some PD models as deleterious in the ability to conclude by increasing variability.

In this PD part, the number of subjects was estimated according to the data of the literature and was driven by the less sensitive test of all the tests planned in this cohort. This less sensitive test was the cough test which requires around 20 subjects to be interpreted.
For this particular model, it is well known that healthy women have a more sensitive cough reflex than healthy men. The reasons for this significant gender difference remain to be elucidated, but may involve a heightened sensitivity, in women, of the sensory receptors within the respiratory tract that mediate cough [2; 3].

In addition, in the apomorphine-induced emesis test, even if 100 % of subjects present emesis after apomorphine injection, the intensity and the number of vomiting episodes could be influenced by the gender (as described in the rotation-induced emesis model which showed gender differences in the unconditioned nausea response to rotation and in the hormonal and the immunological response patterns [4].

Considering these driving factors, and in order to limit the number of subjects exposed in this specific PD exploratory part, only male subjects will be included in the PD exploratory cohort.
This study will be conducted in healthy subjects; therefore, no medical benefit for the subjects can be expected beyond the thorough medical check-up, which each subject will receive prior to treatment and at the end of the study. The potential risks associated with BIA 10-2474 are not known as this product has not yet been tested in humans. As BIA 10-2474 increases exposure to anandamide, endocannabinoid effects, such as catalepsy, hypothermia and hyperphagia, may be potentiated.

Provided the protocol is adhered to, careful observation and medical management will minimize any associated risk in this study.

The participation of healthy subjects in this study is justified with regard to the expected benefit for the target population that will receive this therapy after registration of the compound.

2.1.3.3. Study design

The study will be performed in four parts: the single ascending dose (SAD) part, the food interaction (FI) part, the multiple ascending dose (MAD) part, and a specific PD part.

For discussion of the design of the four parts of this study, please see Section 4.6.

2.1.3.4. Dose selection

No target organ was identified during toxicology studies and few adverse clinical findings were observed at the highest dose tested.

The selection of the single doses to be administered in this study is based on the extrapolation of *in vivo* data from preclinical pharmacologic models, and safety data from the toxicology studies using safety margins derived from the NOAEL.

For the SAD part, a starting dose of 0.25 mg was judged to be safe for a first-in-human administration. Escalation to the next higher dose and any dose adjustments of the next dose levels will be based on safety and tolerability results of the previously administered dose and available PK data of previous dose groups. There will be a 5-fold increase from the first dose level (0.25 mg) to the second dose level. The dose levels of the following groups will be increased by approximately 2-fold from the previous dose level, until the dose exceeds 100 mg, which is the human equivalent dose (HED) corresponding to the NOAEL in the rat. Thereafter, there will be a 50% increase up to the last dose (if applicable). The PK data of SAD Group 1 (Group S1) will be reviewed before the start of Group S2. Further PK data will be available with a lag time of 1 dose (i.e., the PK results of Group S2 will be reviewed before the start of Group S4, etc.). The available PK results may change the planned dose levels. If the maximum tolerated dose (MTD) is not reached after completing the planned sequential groups, additional groups can be included to a maximum of 12 groups.

The dose escalation steps are supported by the preliminary preclinical studies. Depending on the tolerance, this range of doses may be modified during the first-in-man study, and intermediate doses may be added.

For the FI part, the dose will be decided during the SAD part (and the MAD part if available). The BIA 10-2474 dose and the use of a single or multiple dose(s) will be decided based on previous PK data (nevertheless the daily dose will not exceed 33 % of the MTD or the maximum dose given in the SAD part). BIA 10-2474 will be administered in one period under fasting conditions and after a high fat breakfast in the other period.

For the MAD part, dosing is expected to be once daily in the morning, but will depend on the outcome of the previous parts. If necessary, the dosing may be more than once daily and the daily dose will be divided according to the new schedule.

The dose levels for MAD Groups 1 to 4 (Groups M1 to M4) will be determined after evaluation of the safety, tolerability and available PK results of previous SAD and MAD (when applicable) dose groups. The MAD part may start during the SAD part, but only when enough PK and safety data are available. If the MTD is not reached after completing the planned sequential groups, additional groups can be included to a maximum of 8 groups.

The dose escalation may also be decreased based on food effects or anticipated accumulation.

The dosing in the MAD part is expected to be under fasting conditions; however, this should be confirmed by the FI part. If PK data suggest there is a positive effect of food (increase of exposure), the subjects may receive the drug in fed conditions.

If there are drug safety concerns, the subjects' dosing will be staggered (a maximum of 4 subjects dosed on the same day and 24 hours of follow-up necessary before dosing the remaining subjects).

If there is no major safety issue based on new preclinical/clinical data, only the first SAD group will be staggered (in the first dose, 2 subjects (1 verum, 1 placebo) will be dosed 24h before the remaining 6 subjects, and if the safety and tolerability results are acceptable, the 6 remaining subjects (5 verum and 1 placebo) will be dosed. Nevertheless, an interval of 10 minutes between each subject will be respected.

An increase in dose will only occur if the previous dose is demonstrated to be generally safe and tolerable. Safety and tolerability will be evaluated continuously through on-going assessments of Adverse Events (AEs), blood and urine safety labs, ECGs, vital signs and physical examinations.

3. OBJECTIVES

3.1. Primary Objectives

The primary objectives are:

- To assess the safety and tolerability of BIA 10-2474 after single and multiple oral doses
- To investigate the effect of food on the PK and PD of BIA 10-2474

3.2. Secondary Objectives

The secondary objectives are:

- To characterize the PK profile of BIA 10-2474 (and its metabolites) after single and multiple oral doses
- To characterize its PD profile [mainly FAAH activity inhibition but also concentrations of N-arachidonoyl-ethanolamine (anandamide, AEA), and related fatty acid amides (FAAs) concentrations such as PEA (N-palmitoylethanolamide), OEA (N-oleoylethanolamide) and LEA (N-lineleoyl ethanolamide)]
- To assess several potential PD effects.

4. STUDY DESIGN

4.1. Description

This is a double blind, randomised, placebo-controlled combined SAD and MAD study, including an additional FI part (which is an open label study), and a PD part.

4.2. Number of Subjects

One hundred and twenty-eight (128) healthy young subjects (64 in the SAD part, 12 in the FI part, 32 in the MAD part and 20 in the PD part), who volunteer for study participation are planned to be enrolled. If the MTD is not reached after completing the planned SAD and MAD sequential groups, additional groups can be included in the SAD and MAD parts up to a maximum of 96 and 64 subjects, respectively.

Due to the exploratory nature of this investigation, there is no formal statistical hypothesis testing. The sample size is based on empirical considerations and on the literature [5; 6].

4.3. Number of Study Centres

The study will be performed at one investigational site in France: Biotrial Rennes.

4.4. Duration of Participation

The total duration for a single subject in each part is:

SAD: Each subject will participate in the study for approximately 8 weeks. Participation will include a screening evaluation between 3 and 28 days before the first administration, 1 check-in period of approximately 5 days. A follow-up visit will be performed 14 to 21 days after discharge or early discontinuation.

FI: Each subject will participate in the study for approximately 11 weeks. Participation will include a screening evaluation between 3 and 28 days before the first administration, 2 check-in periods of approximately 5 days each, with a wash-out period of at least 14 days between administrations. A follow-up visit will be performed 14 to 21 days after discharge from the last treatment period (Period 2) or early discontinuation.

MAD: Each subject will participate in the study for approximately 9 weeks. Participation will include a screening evaluation between 3 and 28 days before the first administration, 1 check-in period of approximately 14 days. A follow-up visit will be performed 14 to 21 days after discharge or early discontinuation.

PD: Each subject will participate in the study for approximately 13 weeks. Participation will include a screening evaluation between 2 and 28 days before the first administration, 2 check-in periods of approximately 12 days each, with a wash-out period of at least 14 days between administrations. A follow-up visit will be performed 14 to 21 days after discharge from the last treatment period (Period 2) or early discontinuation.

4.5. Study endpoints

4.5.1. Safety endpoints

The safety and tolerability parameters evaluated during this study are:

- Clinical safety (adverse event information, prior and concomitant medications assessment, physical examination (including body weight), vital signs, digital standard 12-lead ECGs).
- Laboratory safety assessments (standard haematology, plasma biochemistry analyses, coagulation, urinalysis, serology, alcohol breath test, urine drug screen).

4.5.2. Pharmacokinetic endpoints

The following parameters for BIAL 10-2474 will be derived, where appropriate:

Plasma:

- Maximum observed plasma concentration (C_{max}),
- Time of occurrence of C_{max} (t_{max}),
- Terminal elimination rate constant (λ_z),
- Terminal half-life ($t_{½}$),
- Area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}),
- Area under plasma concentration-time curve from hour 0 to infinity AUC_{inf},
- Apparent volume of distribution (Vz/F),
- Total body clearance (CL/F),
- Last measurable plasma concentration (C_{last}),
- Time to reach last measurable plasma concentration (t_{last}),
- Area under the plasma concentration-time curve over one dosing interval ($AUC_{0-\tau}$),
- Average plasma concentrations at steady state ($C_{average}$),
- Minimum observed plasma concentration (C_{min}),
- Accumulation ratio of C_{max} (R_{acc} C_{max}),
- Accumulation ratio of $AUC_{0-\tau}$ (R_{acc} $AUC_{0-\tau}$),
- Concentration at the end of a dosing interval before the next dose administration (C_{trough}),
- Peak-trough fluctuation (PTF%).

Urine:

- Renal clearance (CL_r),
- Total amount excreted in urine (A_e),
- Percent of drug recovered in urine ($A_{e\ \%dose}$).

Additional parameters could be calculated if deemed necessary.

4.5.3. Pharmacodynamic endpoints

The following parameters will be calculated, where appropriate, for FAAH activity [expressed as the amount of deuterated ethanolamide (EA-d4) formed by the action of the FAAH in isolated leukocytes samples, on an anandamide substrate]:

- Maximum observed effect on FAAH activity (E_{max}),
- Time to occurrence of E_{max} (t_{Emax}),
- Area under the effect-time curve (AUEC).

Additionally, plasma levels of AEA and related FAAs like PEA, OEA and LEA will also be analysed.
Additional parameters could be calculated if deemed necessary.

The following parameters will be calculated for psychometric tests and scales:

- M-(marijuana) score,
- Number of correct answers in Choice Reaction Time (CRT) and Digit Vigilance (DV),
- Mean response time in CRT, DV and Rapid Visual Information Processing (RVIP),
- Number of correctly detected targets in RVIP,
- Number of missed targets and number of false answers for DV and RVIP,
- Number of words correctly recalled in immediate recall 1, mean number of words correctly recalled in all immediate recalls and number of words recalled in delayed recall in Learning Memory test (LMT),
- Sleepiness score for Stanford Sleepiness Score (SSS) and,
- Tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment scores in Profile of Mood States (POMS).

Additional parameters could be calculated if deemed necessary.

The following parameters will be calculated for cold pressor test:

- Time endured with hand submerged in cold water (cold pressor tolerance-CP_{tol}),
- Maximum value observed on pain Visual Analogue Scale (VAS) (pain VAS_{max}),
- Total of values observed until the withdrawal from the bath (pain VAS_{total}),
- Analogue scale with replacement of missing values between time of withdrawal and 120 seconds by maximum value (100) (pain $AUC_{0\text{-}120s}$).

Additional parameters could be calculated if deemed necessary.

The following parameters will be calculated for cough challenge:

- Concentrations of capsaicin causing five coughs (C5),
- Concentrations of capsaicin causing two coughs (C2),
- Number of coughs during the first 15s after administration for each capsaicin concentration,

- Number of coughs from 15s after to 1 minute after administration for each capsaicin concentration.

Additional parameters could be calculated if deemed necessary.

The following parameter will be measured for intraocular pressure (IOP) measurement:

- IOP (in mmHg).

Additional parameters could be calculated if deemed necessary.

The following parameters will be calculated for apomorphine challenge:

- Maximum degree of nausea (NS) (NS_{max}),
- Time of the maximum degree of nausea (NS t_{max}),
- Area under the degree of nausea versus time curve [NS $AUC_{(0\text{-}90\ mins)}$].

Additional parameters could be calculated if deemed necessary.

4.6. Discussion of the Design

The selected designs for the four parts of this study are as follows:

- The SAD part consists of 8 groups of 8 healthy young male and female subjects, gender balanced to the extent possible, each receiving a single oral dose of BIA 10-2474 or placebo (6 verum and 2 placebos). In the first group, 2 subjects (1 verum and 1 placebo) are to be dosed 24 h before the remaining 6 subjects, and if the safety and tolerability results are acceptable, the remaining 6 subjects (5 verum and 1 placebo) will be dosed. If the MTD is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 12 sequential groups in total.

- The FI part consists of 12 healthy young male and female subjects, gender balanced to the extent possible, each receiving either a single dose or multiple doses of BIA 10-2474 in either the fed or fasting state in an open-label, two-way crossover design. Treatment periods will be separated by at least 14 days (between the last administration of Period 1 and the first administration of Period 2).

- The MAD part consists of 4 groups of 8 healthy young male and female subjects, gender balanced to the extent possible, each receiving an oral dose of BIA 10-2474 or placebo (6 verum and 2 placebos) once daily for 10 days. If the MTD is not reached after completing the planned sequential groups, additional groups can be included up to a maximum of 8 sequential groups in total.

- The PD part consists of 1 group of 20 male subjects performing a double-blind, placebo-controlled, two-way cross-over design to assess the effects of BIA 10-2474 on various PD models (pain, antitussive, anti-emetic) and on psychometric tests and IOP measurement. Treatment periods will be separated by at least 14 days (between the last administration of Period 1 and the first administration of Period 2).

These designs are well-established for first-in-man studies and appropriate to assess the preliminary safety and tolerability of small molecules such as BIA 10-2474.

For the SAD part, the sample size of 8 subjects per dose level (6 verum and 2 placebos) is deemed adequate for this type of study [5].

For the FI part, there will be 12 healthy young subjects. This number is deemed adequate for an initial evaluation of food effect. The wash-out period the and follow-up observation period after Period 2 is anticipated to be 14 days, but the actual duration will take into account the results for PK parameters in the SAD part.

For the MAD part, there will be 8 healthy young subjects per dose level (6 verum and 2 placebos). This number is deemed adequate for the objectives of this study part.

For the PD part, 20 subjects will be enrolled. The review of the literature showed that the recommended sample size for the cough challenge is 20 subjects. The sample sizes used in the other PD models (apomorphine challenge and cold pressor test) are generally smaller than 20 subjects, thus this number is deemed adequate for the objectives of this study part.

For the cough challenge model, it is well known that healthy women have a more sensitive cough reflex than healthy men. The reasons for this significant gender difference remain to be elucidated, but may involve a heightened sensitivity in women of the sensory receptors within the respiratory tract that mediate coughing [2; 3].

In addition, in the apomorphine-induced emesis test, even if 100 % of subjects present emesis after apomorphine injection, the intensity and the number of vomiting episodes could be influenced by the gender (as described in the rotation-induced emesis model, which showed gender differences in the unconditioned nausea response to rotation and in the hormonal and the immunological response patterns [4]).

Considering these factors, and in order to limit the number of subjects exposed in this specific exploratory PD part, only male subjects will be included in the exploratory PD cohort.

During this study, safety parameters will be closely followed by regular measurements, to enable a detection of any changes as early as possible and to be able to stop dosing before any subject sustains major harm. The follow-up observation for the end of study visit will take into account the results of previously available PK parameters (especially for the potential occurrence of AEs) and could be changed if necessary for safety reasons.

The use of placebo as a control is necessary to provide reliable scientific evidence of safety and tolerability and to ensure a reliable evaluation of the pharmacological activity of the tested drug.

A double-blind design is used in conjunction with a placebo control to prevent the evaluation being biased by the expected effects of the test article.

The sequential ascending dose design will be used for a safe determination of the MTD and the establishment of a sufficient safety margin over the expected therapeutic doses in humans.

Evaluation of BIA 10-2474 administered in the fasting and fed states will provide a preliminary assessment of the effect of food on the bioavailability and/or on certain safety and tolerability parameters of BIA 10-2474.

This study design is in line with current regulatory guidance and appropriate to reach the objectives of the study.

4.7. Identification of Source Data

The source data will not be directly collected in the Case Report Form (CRF) but will be captured in supportive documentation (study source documents): laboratory parameters and clinical interpretation of the values on the analytical laboratory print outs, ECG records and clinical significance of observations (when applicable) on the ECG print-outs. The psychometric tests and scales will be computer-assisted assessments; thus the source data will be electronically saved. The other investigated parameters are collected in the Investigator's source data book.

The data collected in the CRF are copied from this source data documentation.

5. STUDY POPULATION

Subjects must give written informed consent for participation in the study before any study-specific screening tests or evaluations.
Nevertheless, if necessary and after documented agreement with the sponsor, any measures identical to those planned in the protocol for the subjects' screening and already performed within the timelines given by the protocol for the screening exams (and after a Biotrial generic written informed consent) could be used for the protocol in order to minimize the constraints on the subjects.

5.1. Inclusion criteria

Subjects must satisfy all of the following inclusion criteria before being allowed to participate/continue in the study:

1. Male or female subjects (only male subjects for the PD part) aged 18 to 55 years, inclusive;
2. Body mass index (BMI) between 19 and 30 kg/m^2 inclusive;
3. Healthy as determined by pre-study medical history, physical examination, vital signs, complete neurological examination and 12-lead ECG;
4. Negative tests for Hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV) and human immunodeficiency virus (HIV)-1 and HIV-2 antibody at screening;
5. Clinical laboratory test results clinically acceptable at screening and admission;
6. Negative screen for alcohol and drugs of abuse at screening and admission;
7. Non-smokers or ex-smokers (must have ceased smoking >3 months prior screening visit);

<u>If female:</u>

8. Woman with no childbearing potential by reason of surgery or at least 1 year post-menopause (i.e., 12 months post last menstrual period), or menopause confirmed by follicle-stimulating hormone (FSH) testing;
9. If of childbearing potential, using an effective nonhormonal method of contraception (intrauterine device or intrauterine system; condom or occlusive cap [diaphragm or cervical or vault caps] with spermicidal foam or gel or film or cream or suppository; true abstinence; or vasectomized male partner, provided that he is the sole partner of that subject) for all the duration of the study and up to one month after the last investigational medicinal product (IMP) administration;
10. Negative serum pregnancy test at screening and negative urine pregnancy test on admission of each treatment period (women of childbearing potential only);

<u>If male:</u>

11. Using an effective method of contraception (condom or occlusive cap [diaphragm or cervical or vault caps] with spermicidal foam or gel or film or cream or suppository; true abstinence; or vasectomy) throughout the study and up to one month after the last IMP administration.

<u>For the PD part only:</u>

12. Normal forced expiratory volume in one second (FEV1) at screening (80-120% predicted for the best FEV1 of 3 measures with a difference between the two best measures < or equal to 0.150 L);
13. Responsive (within acceptable ranges) to the cough provocation agent (capsaicin);
14. Normal basal IOP (between 10-20 mmHg inclusive);
15. At screening, a cold pressor tolerance ≥15 seconds and ≤120 seconds on three measurements, after one training trial, with a deviation ≤20 seconds between the three measurements

5.2. Exclusion criteria

If any of the following exclusion criteria apply, the subject must not enter/continue in the study:

1. Subjects who have a clinically relevant history or presence of respiratory, gastrointestinal, renal, hepatic, haematological, lymphatic, neurological, cardiovascular, psychiatric, musculoskeletal, genitourinary, immunological, dermatological, endocrine, connective tissue diseases or disorders;
2. Have a clinically relevant surgical history;
3. Have a history of hyperemesis;
4. Have a history of relevant atopy or drug hypersensitivity;
5. Have a history of alcoholism or drug abuse;
6. Consume more than 14 units of alcohol a week [1 glass (25 cl) of beer with 3° of alcohol = 7.5 g, or 1 glass (25 cl) of beer with 6° of alcohol = 15 g, or 1 glass (12.5 cl) of wine with 10° of alcohol = 12 g, or 1 glass (4cl) of aperitif with 42° of alcohol = 17 g];
7. Have a significant infection or known inflammatory process on screening or admission;
8. Have acute gastrointestinal symptoms (e.g., nausea, vomiting, diarrhea, heartburn) at the time of screening or admission;
9. Have used medicines within 2 weeks of admission that may affect the safety or other study assessments, in the investigator's opinion;
10. Have used any investigational drug or participated in any clinical trial within 90 days prior to screening;
11. Have participated in more than 2 clinical trials within the 12 months prior to screening;
12. Have donated or received any blood or blood products within the 3 months prior to screening;
13. Are vegetarians, vegans or have medical dietary restrictions;
14. Cannot communicate reliably with the investigator;
15. Are unlikely to co-operate with the requirements of the study;
16. Are unwilling or unable to give written informed consent.

<u>If female:</u>

17. Pregnancy or breast-feeding;
18. Woman of childbearing potential not using an accepted effective contraceptive method or using oral contraceptives;

<u>If male:</u>

19. Not using an accepted effective method of contraception.

5.3. Withdrawal criteria and related procedures

5.3.1. Withdrawal criteria

Any subject may be withdrawn from the study at the discretion of the Investigator. The subject is also free to terminate his participation at any time. However, if the subject has been dosed with study medication, it is recommended to ask the subject to remain in contact with the centre and the investigational site should make every effort to convince the subject to return for a safety follow-up visit. The safety follow-up visit will then be organised.

The Investigator also undertakes to obtain more detailed information about any subject lost to follow-up.

Subjects withdrawn from the study must not be re-included.

5.3.2. Withdrawn subject data collection

The Principal Investigator and/or another Investigator involved in the study will document on the termination page of the CRF and in the subject's medical records the primary reason for the subject's withdrawal as follows:

- Lost to follow-up: subjects who leave the clinic during the hospitalisation period or do not attend the following visit. Intensive efforts should be made to locate and recall them if possible and to determine their health status at a minimum.
- AE: an AE form must be completed.
- Deviation from protocol.
- Withdrawal of consent: in this case, it should be clarified with the subject if the underlying reason for withdrawal is an AE or not. This clarification should be documented.
- Other: if none of the above-mentioned reasons are applicable, then the reason will be specified.

All withdrawals will be reported to the Sponsor.

For all drop-outs, the safety follow-up visit will be arranged 10 to 14 days after the drop-out day, and will document the progress of their condition. In every case, the CRF must be filled in up to the last visit performed.

5.3.3. Replacement of subjects

Subjects who withdraw or drop out may be replaced. The replacement subject will be assigned to the same treatment or treatment sequence as the replaced subject.

If a subject is withdrawn from the study due to an AE, the Investigator should follow the procedures documented in Section 13 to assess the safety of the IMP.

6. STUDY DRUGS

6.1. Description of treatment

6.1.1. BIA 10-2474

A detailed description of BIA 10-2474 can be found in the IMP dossier (IMPD), Drug Substance module.

- **Description of the dosage form:**

BIA 10-2474 will be supplied as 0.25, 2.5 and 10 mg light blue hard gelatine capsules. Subject doses will be composed of a single strength or combinations of different strengths.

- **Composition:**

The capsules are composed of the respective amount of active ingredient. The drug-in-capsule approach is used to prepare the study medication.

6.1.2. Placebo

- **Description of the dosage form:**

Placebo will be supplied as hard gelatine capsules matching the appearance and other visual and organoleptic characteristics of BIA 10-2474 capsules.

6.1.3. Provocation agents

6.1.3.1. Capsaicin solution

- **Description of the dosage form:**

Capsaicin will be supplied by Baccinex and released by LC2 as unidoses of sterile 3.33mg/ml capsaicin solution.

Fresh dilutions will be made from this stock solution in 0.9% saline solution on a daily basis in order to obtain serial doubling concentrations ranging from 0.9765 to 500 µmol/L, with the knowledge that, in the literature, induction of cough is rare at the lowest concentration.

To increase challenge blindness, inhalation of 0.9% saline solution will be randomly interspersed.

For each step of concentration, an adequate volume of diluted capsaicin solution or 0.9% saline solution will be placed in a breath-activated nebuliser with a dosimeter which will deliver 20µL of solution per puff.

Each volunteer participating to this cough challenge assessment will test until 10 concentrations of capsaicin to determine the concentration causing two and five coughs at screening (=baseline) and on Day 7 of each period.

All instructions for the reconstitution will be available in the corresponding Pharmacy Manual.

- **Composition:**

The components of capsaicin are shown in Table 1.

Table 1: Composition of capsaicin solution

Ingredients	Function	Unit formula per vial 250µL)	Reference to Standards
Capsaicin + Dihydrocapsaicin	Net Drug Substance	0.833 mg	USP
Tween 80	Solvent	18.75 mg	Ph.Eur.
Sodium chloride	Diluent	2.25 mg	Ph. Eur
Sodium chloride injection 0.9% (saline)	Diluent	Qs. 250 µL	Ph.Eur.

6.1.3.2. Apomorphine

- **Description of the dosage form:**

Apomorphine will be supplied by Biotrial as a marketed injectable dosage form of apomorphine 5 mg/mL (Apokinon®).

A volume of apomorphine 5 mg/mL will be transferred in a sterile syringe to obtain a quantity of 50 µg/kg for each subject.

All instructions for the reconstitution will be available in the corresponding Pharmacy Manual.

- **Composition:**

The components of apomorphine are shown in Table 2.

Table 2: Composition of apomorphine

Active ingredient	Apomorphine chlorhydrate 50 mg/vial
Excipients	Sodium metabisulfite (E223), concentrated hydrochloric acid for pH adjustment, water for injectable preparations

6.2. Dose administration

All treatments will be administered orally.

SAD

Group S1: A single dose of 0.25 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S2: A single dose of 1.25 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S3: A single dose of 2.5 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S4: A single dose of 5 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S5: A single dose of 10 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S6: A single dose of 20 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S7: A single dose of 40 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1
Group S8: A single dose of 100 mg BIA 10-2474 (n=6) or matching placebo (n=2) on D1

If the MTD is not reached after completing the planned sequential groups, additional groups can be included to a maximum of 12 groups.

FOOD INTERACTION

Group FI: The dose design of the food interaction will be decided during the SAD part (and MAD part if available). The BIA 10-2474 dose and the use of a single or multiple dose(s) will be decided based on the previous PK data (nevertheless the daily dose will not exceed 33% of the MTD or the maximum dose given in the SAD part). BIA 10-2474 will be administered in one period under fasting conditions and after a high fat breakfast in the other period. These will be administered in a randomized order according to a cross-over design.

MAD

Groups M1 to M4: Multiple doses of BIA 10-2474 or matching placebo once daily from D1 to D10. The dose levels of Groups M1 to M4 will be determined after evaluation of the safety, tolerability and available PK results of previous SAD and MAD (when applicable) dose groups. The MAD part may start during the SAD part but only when enough PK and safety data are available.

If the MTD is not reached after completing the planned sequential groups, additional groups can be included to a maximum of 8 groups.

PD

Group PD: Repeated dosing from D1 to at least D10 with placebo and a well-tolerated BIA 10-2474 dose, chosen after analysis of previous SAD/MAD cohorts. These will be administered in a randomized order according to a cross-over design. The duration of each repeated dose is to be decided according to previous SAD and MAD PK results.

Product batches used will be identified in the final Clinical Study Report (CSR).

IMPs will be given orally with 240 mL of water early in the morning after an 8-hour overnight fast in each period. Note: As the 100 mg SAD dose implies to take 10 capsules of 10 mg, the volume of water can be increased to 270 mL if 240 mL is not sufficient.

Detailed dose escalation and stopping rule procedures are set out in Section 9.2.

6.3. Supply, packaging and labelling of the investigational products

The IMPs (BIA 10-2474 and placebo capsules) will be bulk supplied by BIAL in high-density polyethylene (HDPE) containers and delivered to the investigational site.

A sufficient quantity of the IMPs will be supplied to Biotrial to perform the study, and Biotrial will complete an acknowledgement of receipt form to be returned to the Sponsor. The Sponsor's representative will provide a certificate of analysis and a QP batch release for those batches of IMP to be used in the study.

The IMPs and the challenge agents (capsaicin and apomorphine) will be packed and labelled per subject at Biotrial according to the European Union-Good Manufacturing Practice (EU-GMP), including its annex 13, and all local regulations.

The investigational products will bear at least the following information on the labels:

- Sponsor's name, address and telephone number
- Study code
- Study drug description and content

- Route of administration
- Identification of subject
- Dosage form
- Randomisation number, Visit number
- Investigator's name
- Batch and/or code number
- Expiry date or Retest date
- Storage conditions
- Instructions for use
- Phrase "For biomedical research only"

The Investigator, or his designee, will only dispense IMPs to subjects included in this study. Each subject will only be given the IMP carrying his number. The dispensing for each subject will be documented in the subject's CRF.

6.4. Storage of the investigational products

IMPs and apomorphine vials will be stored at ambient temperature (do not freeze, store below 25°C).

Capsaicin vials will be stored in a cold room (store between 2 and 8°C).

All IMPs and challenge agents will be stored in a secure and locked storage area with limited access, with temperature control and monitoring.

The pharmacist at Biotrial will be responsible for the correct storage and handling of the IMPs and challenges agents.

Any deviations from the storage requirements, including actions taken, must be documented and communicated to the Sponsor in advance of any eventual actions.

6.5. Accountability, reconciliation and return of the investigational products

The Investigator must maintain a complete and current dispensing and inventory record. The Investigator is accountable for all test articles supplied by the Sponsor. The dispenser will use this information to maintain an accurate and complete dispensing and inventory record. The designated copies of the completed dispensing and inventory record will be returned to the Sponsor.

Regulatory agencies require accounting for the disposition of all investigational drugs received by each clinical site. Information on drug disposition required by law consists of the date received, date administered, quantity administered, and the subject to whom the drug was administered.

All unused IMPs must be returned in the original containers. Non-used, returned study drug and empty IMP containers may be destroyed after the Pharmacist and the monitor have performed accountability and the Sponsor has confirmed in writing that destruction can occur.

Supplies are shipped to the investigational site as needed. Drug accounting will be reviewed by the site monitor during routine monitoring visits. Each time a dose is dispensed to a subject, the following information should be recorded: the subject's study number, the total dose dispensed, the number of capsules used, the batch number, and the initials of the person dispensing the dose.

At the completion or termination of the study, a final drug accountability review and reconciliation must be completed, and any discrepancies must be investigated and their resolution documented.

6.6. Treatment compliance

All IMP doses will be administered in the clinical unit under the direct supervision of the Investigator or his designee.

After administration of IMP, a hand check and a mouth check will be performed to verify that the subject has swallowed the dose.

Drug administration information will be recorded in the CRF and in the drug accountability form.

7. PRIOR TREATMENTS

Reasonable efforts will be made to determine all relevant treatments received by the subject within 2 weeks before IMP administration. All relevant information must be recorded on the subject's CRF.

8. CONCOMITANT TREATMENTS

As a general rule, no concomitant medication will be permitted, with the exception of medications to treat AEs, unless the rationale for the exception is discussed and clearly documented between the Investigator and the Sponsor.

9. PROCEDURES

9.1. Investigational schedule

Flow chart 1 summarises the schedule of assessments in the SAD part; Flow chart 2 summarises the schedule of assessments in the FI part; Flow chart 3 summarises the schedule of assessments in the MAD part; Flow chart 4 summarises the schedule of assessments in the PD part.

Before dosing and in order to have enough time for breakfast and/or PD predose assessments, when several procedures are scheduled at the same time, the 12-lead ECG will be obtained first within 60 minutes of the scheduled time, then vital signs will be measured, then the PK blood samples will be collected at the scheduled time within the windows noted in Section 9.3.

After dosing, when several procedures are scheduled at the same time, the 12-lead ECG will be obtained first within 15 minutes of the scheduled time, then vital signs will be measured, then the PK blood samples will be collected at the scheduled time within the windows noted in Section 9.3.

9.1.1. Screening Visit (between D-28 and D-3; between D-28 and D-2 in PD part)

The eligibility criteria will be documented on the CRF. The subject will be provided with all information regarding the study objectives and procedures from the Investigator. The subject will be assigned a screening number after signing the informed consent form (ICF).

Adverse events will be monitored throughout the study.

The screening visit will include the following:

- Signature and date of an ICF before any study specific screening procedures are performed,
- Verification of the eligibility criteria,
- Medical history,
- Physical examination including weight (kg) and height (cm). BMI will be calculated to ensure eligibility,
- Complete neurological examination,
- Vital signs measurement (including tympanic body temperature),
- 12-lead ECG,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation and urinalysis), viral serology testing (includes HBsAg, anti-HCV antibodies, anti-HIV-1 antibodies and anti-HIV-2 antibodies), and a urine drug of abuse screening,
- Alcohol breath test,
- Hormone panel [FSH testing] (if necessary),
- Serum pregnancy test (only for female subjects of childbearing potential),
- Cold pressor test (only in PD part),
- Cough challenge (only in PD part),

- IOP measurement (only in PD part),
- Spirometry (only in PD part).

9.1.2. Admission Visit (D-2, two days prior to dosing; D-1, one day prior to dosing in PD part)

Subjects will be admitted to the unit two days prior to dosing or one day prior to dosing (in the PD part) for each study part (and for each of the two study periods when applicable), and will undergo the following investigations:

- Verification of the eligibility criteria,
- Medical history update,
- Physical examination update,
- Vital signs measurement (including tympanic body temperature),
- 12-lead ECG,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation) only in PD part,
- Urinalysis,
- Urine drug screen,
- Alcohol breath test,
- AE monitoring,
- Urine pregnancy test (only for female subjects of childbearing potential),
- Psychometric scales and tests (only in PD part).

Subjects will be offered a meal, and will be fasting until the following day for a required duration of at least 8 hours in SAD part, PD part and FI part fasting period. The dosing during the MAD part is expected to be also under fasting conditions; however this should be confirmed by the FI part.

9.1.3. SAD part

9.1.3.1. Day before dosing (D-1)

The following procedures will be performed:

- Laboratory evaluation (haematology, plasma biochemistry, coagulation),
- Vital signs in triplicate (without body temperature),
- 12-lead ECG in triplicate,
- PD blood sampling,
- Psychometric scale (M Scale – Marijuana),
- AE monitoring.

9.1.3.2. Dosing Day (D1)

The subjects will be randomised to the group treatment allocated on D1 morning.

The study drug will be administered after an overnight fast of at least 8 hours.

The intake hour (H0) on D1 will be the reference time for implementing the following investigational procedures consistently.

Subjects will have the following procedures performed at pre-dose:

- Verification of the eligibility criteria,
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- M-scale,
- Telemetry,
- AE monitoring.

After drug administration, the following examinations will be performed at several time-points (see Flow chart 1):

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- M-scale,
- Telemetry,
- AE monitoring.

Meals will be served around H4 post-dose for lunch and around H12 post-dose for dinner, after the completion of all examinations.

9.1.3.3. Day 2 (D2)

The following procedures will be performed at several time-points (see Flow chart 1):

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- Telemetry,
- AE monitoring.

A breakfast will be offered after the H24 post-dose sampling has been drawn.

9.1.3.4. Day 3 (D3)

The following procedures will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- AE monitoring.

9.1.3.5. Day 4 (D4): 72 h PK and PD samples and Discharge Visit

The following procedures will be performed:

- Vital signs (including tympanic body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis),
- AE monitoring.

After medical authorization, the subject will be discharged.

The subject will be asked to return for a follow-up visit, scheduled between 14 and 21 days after Day 4 or early discontinuation.

9.1.4. FI part

9.1.4.1. Day before dosing (D-1)

The following procedures will be performed at several time-points (see Flow chart 2):

- Vital signs in triplicate (without body temperature),
- 12-lead ECG in triplicate,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation),
- PD blood sampling,
- AE monitoring.

9.1.4.2. Dosing Day (D1)

The subjects will be randomised to the group treatment allocated on D1 morning of the first period.

The study drug will be administered after an overnight fast of at least 8 hours in the fasting period and 30 minutes after the beginning of a standardized high-fat breakfast in the fed period.

The intake hour (H0) on D1 will be the reference time for implementing the following investigational procedures consistently.

Subjects will have the following procedures performed on D1 of each of the two study periods at pre-dose:

- Verification of the eligibility criteria,
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PD blood sampling.

After drug administration, the following examinations will be performed at several time points (see Flow chart 2):

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PD blood sampling,
- AE monitoring.

In the fed period, a standard breakfast will be offered after the H24 post-dose sampling has been drawn. In the fasting period, subjects will remain fasted for at least 4 hours after dosing.

Meals will be served around H4 post-dose for lunch and around H12 post-dose for dinner, after the completion of all examinations.

9.1.4.3. Day 2 (D2)

The following procedures will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PD blood sampling,
- AE monitoring.

A standard breakfast will be offered after the H24 post-dose sampling has been drawn.

9.1.4.4. Day 3 (D3)

The following procedures will be performed:

- 12-lead ECG,
- PK blood sampling,
- PD blood sampling,

- AE monitoring.

A standard breakfast will be offered after the H48 post-dose sampling has been drawn.

9.1.4.5. Day 4 (D4): 72 h PK and PD samples and Discharge Visit

The following procedures will be performed:

- Vital signs (including tympanic body temperature),
- 12-lead ECG,
- PK blood sampling,
- PD blood sampling,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis),
- AE monitoring.

A standard breakfast will be offered after the H72 post-dose sampling has been drawn.

After medical authorization, the subject will be discharged and be asked to return for Period 2 after a wash-out period of at least 14 days beginning from the IMP administration.

Following the second treatment period, the subject will be asked to return for a follow-up visit, scheduled between 14 and 21 days after Day 4 or early discontinuation.

9.1.5. MAD part

9.1.5.1. Day before dosing (D-1)

The following procedures will be performed at several time-points (see Flow chart 3):

- Vital signs in triplicate (without body temperature),
- 12-lead ECG in triplicate,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation),
- PD blood sampling,
- AE monitoring.

9.1.5.2. Dosing Day (D1)

The subjects will be randomised to the group treatment allocated on D1 morning.

The intake hour (H0) on D1 will be the reference time for implementing the following investigational procedures consistently.

Subjects will have the following procedures performed at pre-dose:

- Verification of the eligibility criteria,
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,

- PD blood sampling,
- Telemetry,
- AE monitoring.

After drug administration, the following examinations will be performed at several time points (see Flow chart 3):

- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- Telemetry,
- AE monitoring.

9.1.5.3. Day 2 (D2)

Before drug administration, the following procedures will be performed

- Telemetry (until T24h post first dose),
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling.

After drug administration, the following examinations will be performed:

- AE monitoring.

A breakfast will be offered after the H24 post-dose sampling has been drawn.

9.1.5.4. Day 3 (D3) and Day 4 (D4)

The following procedures will be performed:

- Drug administration,
- PK blood sampling on D4 only,
- PD blood sampling on D4 only,
- AE monitoring.

9.1.5.5. Day 5 (D5)

Before drug administration, the following examinations will be performed:

- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis).

After drug administration, the following examinations will be performed:

- AE monitoring.

9.1.5.6. Day 6 (D6) to Day 9 (D9)

The following procedures will be performed:
- Drug administration,
- PK blood sampling on D6 and D8 only,
- PD blood sampling on D6 and D8 only,
- AE monitoring.

9.1.5.7. Day 10 (D10)

After the last drug administration, the following examinations will be performed at several time-points (see Flow chart 3):
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- Telemetry (from D10 pre-dose),
- AE monitoring.

9.1.5.8. Day 11 (D11)

The following examinations will be performed:
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling
- Telemetry (until T24h post D10 dose),
- AE monitoring.

9.1.5.9. Day 12 (D12)

The following examinations will be performed at several time-points (see Flow chart 3):
- Vital signs (without body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- AE monitoring.

9.1.5.10. Day 13 (D13): 72 h (D10) PK and PD samples and Discharge Visit

The following procedures will be performed:

- Vital signs (including tympanic body temperature),
- 12-lead ECG,
- PK blood sampling,
- PK urine sampling,
- PD blood sampling,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis),
- AE monitoring.

After medical authorization, the subject will be discharged.

The subject will be asked to return for a follow-up visit, scheduled between 14 and 21 days after discharge or early discontinuation.

9.1.6. PD part

D-1 procedures are described in Section 9.1.2 (admission visit).

Vital signs (without body temperature) and 12-lead ECG will be performed in triplicate.

9.1.6.1. Dosing Day (D1)

The subjects will be randomised to the group treatment allocated on D1 morning of the first period.

The study drug will be administered after an overnight fast of at least 8 hours.

The intake hour (H0) on D1 will be the reference time for implementing the following investigational procedures consistently.

Subjects will have the following procedures performed at pre-dose:

- Verification of the eligibility criteria,
- Vital signs (without body temperature),
- 12-lead ECG,
- Psychometric tests and scales.

After drug administration, the following examinations will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- Psychometric tests and scales,
- AE monitoring.

9.1.6.2. Day 2 (D2)

After randomization and drug administration, the following examinations will be performed:

- Vital signs (without body temperature),

- 12-lead ECG,
- AE monitoring.

A breakfast will be offered after the H24 post-dose sampling has been drawn.

9.1.6.3. Day 3 (D3) and Day 4 (D4)

The following procedures will be performed:

- Drug administration,
- AE monitoring.

9.1.6.4. Day 5 (D5)

After drug administration, the following examinations will be performed:

- Psychometric tests and scales (at t_{max}),
- AE monitoring.

9.1.6.5. Day 6 (D6)

After drug administration, the following examinations will be performed:

- Cold pressor test (at t_{max}),
- AE monitoring.

9.1.6.6. Day 7 (D7)

After drug administration, the following examinations will be performed:

- Cough challenge (at t_{max}),
- AE monitoring.

9.1.6.7. Day 8 (D8)

After drug administration, the following examinations will be performed:

- IOP measurement (at t_{max}),
- AE monitoring.

9.1.6.8. Day 9 (D9)

After drug administration, the following examinations will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- Psychometric tests and scales (at t_{max}),
- AE monitoring.

9.1.6.9. Day 10 (D10)

After the last drug administration, the following examinations will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- Apomorphine challenge (at t_{max}),
- AE monitoring.

9.1.6.10. Day 11 (D11)

The following examinations will be performed:

- Vital signs (without body temperature),
- 12-lead ECG,
- AE monitoring.

9.1.6.11. Day 12 (D12): Discharge Visit

The following procedures will be performed:

- Physical examination,
- Complete neurological examination,
- Vital signs (including tympanic body temperature),
- 12-lead ECG,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis),
- AE monitoring.

After medical authorization, the subject will be discharged.

The subject will be asked to return for a follow-up visit, scheduled between 14 and 21 days after Day 12 or early discontinuation.

9.1.7. Follow-Up / End of Study Visit (for all study parts)

- Vital signs measurement,
- Physical examination update,
- Medical history update,
- 12-lead ECG,
- Laboratory evaluation (haematology, plasma biochemistry, coagulation, and urinalysis),
- Serum pregnancy test (only for female subjects of childbearing potential),
- AE monitoring.

9.2. Dose escalation and stopping rules

The starting dose will be 0.25 mg BIA 10-2474 in Group S1 (SAD part). The dose levels of the following groups will be increased by approximately 2-fold from the previous dose level (except for a 5-fold increase from 0.25 mg to the second dose), until the dose exceeds 100 mg BIA 10-2474, being the HED, corresponding to the NOAEL in the rat. Thereafter, there will be a 50% increase up to the last dose (if applicable). If the MTD is not reached after completing the planned sequential groups, additional groups can be included to a maximum of 12 groups.

The decision to progress to the next higher dose will be made after the safety and tolerability data (treatment emergent adverse events (TEAEs), vital signs, ECG and routine lab tests) are reviewed for each dose group, for 6 out of 8 subjects in the cohort through H48 after (last) dosing. This decision will be made jointly by the Investigator, medical director or representative, and medical monitor or representative of the Sponsor. PK data of Group S1through H 72 after (last) dosing will be reviewed before the start of Group S2. Further PK data through H 72 after (last) dosing (except if available PK data allow the review through H24 or H48 after (last) dosing) will be available with a lag time of 1 dose (i.e., PK results of Group S2 will be reviewed before the start of Group S4, etc.). The available PK results may change the planned dose levels.

The dose should not be escalated further if one of the circumstances listed below occurs in subjects within the same cohort, unless it is obvious that the occurrence is not related to the administration of the treatment:

- Drug related severe AEs of the same character in 4 or more subjects;
- Clinically significant drug-related laboratory abnormalities of the same character in 6 or more subjects [e.g., alanine aminotransferase (ALT) > 3 times the upper limit of normal (3 ULN)].
- Clinically significant drug-related changes in vital signs of the same character in 6 or more subjects [e.g., systolic BP consistently greater than 160 mmHg associated with an increase from baseline greater than 20 mmHg].
- Clinically significant drug-related changes in ECGs of the same character in 6 or more subjects: QT interval corrected for heart rate according to Fridericia (QTcF) greater than 500 msec (confirmed by a repeat test) with an increase from baseline greater than 60 msec, or other abnormalities.

It should be made clear that these are guidelines only, and the Investigator, together with the Sponsor's medical monitor and clinical pharmacologist can make an exception, if justified. However, when such an exception is made, the reasons for it should be clearly documented.
Although these are the minimum criteria, dose escalation may be suspended on the basis of other safety information considered to pose a risk to subjects.
Planned dose escalation may be modified to include the repetition of a dose based on the results of the safety and tolerability review, or if further characterization of the safety profile at that dose is required. Intermediate dose levels or a lower increment can also be done.

9.3. Total volume of blood collected

Blood samples will be obtained according to the following schedule (see Table 3 for SAD, Table 4 for FI, Table 5 for MAD, and Table 6 for PD):

Table 3: SAD part: Blood Volume and Collection Schedule

Screening Visit (D-28 to D-3)	
Biochemistry*, Haematology, Coagulation, Serology*, Serum Pregnancy* (if applicable), FSH (if applicable)	3.5+3+2 + 3.5[#] mL ***Total =12 mL***
Admission Visits (D-2 & D-1)	
Biochemistry, Haematology, Coagulation, PD samples	3.5+3+2+(9 samples x 4) mL ***Total = 44.5 mL***
Dosing Visit (D1)	
PK samples, PD samples	(9 samples x 3)+(9 samples x 6)+(9 samples x 4) mL ***Total = 117 mL***
D2	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
D3	
PK samples, PD samples	3+6+4 mL ***Total = 13 mL***
D4 Discharge	
Biochemistry, Haematology, Coagulation, PK sample, PD sample	3.5+3+2+3+6+4 mL ***Total = 21.5mL***
Follow up visit	
Biochemistry*, Haematology, Coagulation, Serum Pregnancy* (if applicable)	3.5+3+2 mL ***Total = 8.5 mL***
Overall duration of the study	**Total = 229.5 mL**

**Biochemistry, serology and serum pregnancy assessments are performed on the same blood sample.*

[#]FSH levels measured in post-menopausal female subjects only if less than12 months post last menstrual period.

Table 4: FI part: Blood Volume and Collection Schedule

Screening Visit (D-28 to D-3)	
Biochemistry*, Haematology, Coagulation, Serology*, Serum Pregnancy* (if applicable), FSH (if applicable) *These are performed on the same blood sample	3.5+3+2 + 3.5[#] mL ***Total =12 mL***
Admission Visit (D-1)	
Biochemistry, Haematology, Coagulation, PD samples	3.5+3+2+ (9 samples x 4) mL per period ***Total = 44.5 mL x 2=89 mL***
Dosing Visit (D1)	
PK samples, PD samples	(9 samples x 3)+(9 samples x 4) mL per period ***Total = 63 mL x 2= 126 mL***
D2	
PK samples, PD samples	3+4 mL/sample per period ***Total =7 mL x 2= 14 mL***
D3	
PK samples, PD samples	3+4 mL/sample per period ***Total =7 mL x 2= 14 mL***
D4 Discharge	
Biochemistry, Haematology, Coagulation, PK samples, PD samples	3.5+3+2+3+4 mL/sample per period ***Total =15.5 mL x 2= 31 mL***
Follow up visit	
Biochemistry*, Haematology, Coagulation, Serum Pregnancy* (if applicable)	3.5+3+2 mL ***Total = 8.5 mL***
Overall duration of the study	**Total = 294.5 mL**

**Biochemistry, serology and serum pregnancy assessments are performed on the same blood sample.*

[#]*FSH levels measured in post-menopausal female subjects only if less than12 months post last menstrual period.*

Table 5: MAD part: Blood Volume and Collection Schedule

Screening Visit (D-28 to D-3)	
Biochemistry*, Haematology, Coagulation, Serology*, Serum Pregnancy* (if applicable), FSH (if applicable)	3.5+3+2 + 3.5[#] mL for FSH ***Total =12 mL***
Admission Visit (D-1)	
Biochemistry, Haematology, Coagulation, PD samples	3.5+3+2+(9 samples x4) mL ***Total = 44.5 mL***
Dosing Visit (D1)	
PK samples, PD samples	(9 samples x 3) + (9 samples x 6) + (9 samples x 4) mL ***Total = 117 mL***
Dosing visit (D2)	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
Dosing visit (D4)	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
Dosing visit (D5)	
Biochemistry, Haematology, Coagulation	3.5+3+2mL/sample ***Total = 8.5 mL***
Dosing visit (D6)	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
Dosing visit (D8)	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
Dosing visit (D10)	
PK samples, PD samples	(9 samples x 3) + (9 samples x 6) + (9 samples x 4) mL ***Total = 117 mL***
D11	
PK samples, PD samples	3+6+4 mL ***Total =13 mL***
D12	
PK samples, PD samples	(2 samples x 3) + (2 samples x 6) + 4 mL ***Total =22 mL***
D13 Discharge	
Biochemistry, Haematology, Coagulation, PK samples, PD samples	3.5+3+2+3+6+4 mL ***Total = 21.5 mL***
Follow up visit	
Biochemistry*, Haematology, Coagulation, Serum Pregnancy (if applicable)*	3.5+3+2 mL ***Total = 8.5 mL***
Overall duration of the study	**Total = 416 mL**

**Biochemistry, serology and serum pregnancy assessments are performed on the same blood sample.*

[#]FSH levels measured in post-menopausal female subjects only if less than12 months post last menstrual period.

Table 6: PD part: Blood Volume and Collection Schedule

Screening Visit (D-28 to D-2)	
Biochemistry, Haematology, Coagulation, Serology	3.5+3+2 mL ***Total =8.5 mL***
Admission Visit (D-1)	
Biochemistry, Haematology, Coagulation	3.5+3+2 mL per period ***Total = 8.5 mL x 2=17 mL***
D12 Discharge	
Biochemistry, Haematology, Coagulation	3.5+3+2 mL per period ***Total = 8.5 mL x 2=17 mL***
Follow up visit	
Biochemistry, Haematology, Coagulation	3.5+3+2 mL ***Total = 8.5 mL***
Overall duration of the study	**Total = 51 mL**

Blood samples for PK and PD analyses should be collected at the requested times; however, a window of ± 10%, but no more than 15 minutes, for sample collection will be allowed. The exact actual time of collection should be noted in the CRFs and used for the PK calculations.

Less than 500 mL of blood (which corresponds approximatively to a blood donation) will be collected during the whole study.

9.4. Prohibitions and restrictions applying to subjects

Tobacco use is prohibited.

Subjects will be requested to abstain from strenuous physical activity, consumption of grapefruit or grapefruit-containing products, alcohol and stimulating beverages containing xanthine derivatives (i.e., no coffee, tea, chocolate or cola like drinks) for 48 hours prior to admission and until the follow up visit.

Meals will be provided during the subjects' stay in the clinic. Drinking of water will be allowed *ad libitum* except for 1 h before and 1 h after dosing.

SAD and PD:
An overnight fast will be imposed so that no food is taken within at least 8 hours before IMP administration. Subjects will remain fasted for at least 4 hours after dosing.

MAD:

The dosing of MAD is expected to be under fasting conditions; however this should be confirmed by the FI part.

FI:

Fasting period: An overnight fast will be imposed so that no food is taken within at least 8 hours before IMP administration. Subjects will remain fasted for at least 4 hours after dosing.

Fed period: On PK days, IMP will be administered 30 minutes after start of intake of a standardized high-fat breakfast. Composition of the standardized high-fat breakfast is 2 eggs fried in butter, 80 g of bacon, 40 g of toast, 25 g of butter, 120 g of potatoes and 240 mL of whole milk (i.e., 180 protein, 220 carbohydrate and 600 fat calories).

10. MEASURES TO MINIMIZE BIAS

10.1. Randomisation procedure

The randomization schedule for each part and three sets of individual sealed codebreak envelopes (for the SAD, MAD and PD parts) will be generated by a Biotrial Statistician, who will be different from the study statistician, using SAS software.

One copy of the randomization list will be provided to the unblinded pharmacist for preparation of the treatment for the SAD, FI, MAD and PD parts.

One set of the sealed codebreak envelopes will be provided to the investigational site, one sent to the Sponsor and one sent to the pharmacovigilance (PV) contact for the trial.

No set of sealed codebreak envelopes will be prepared for the FI part as this part will be open-label.

Subjects withdrawn from the study retain their randomization number, if already given. New subjects must always be allotted a new randomization number/treatment number (replacement treatment number): once assigned, randomization numbers are never re-used within the study site. Note that subjects will be identified by their randomization numbers, throughout the entire course of the study.

Subjects who withdraw or drop out could be replaced. The replacement subject will be assigned to the same treatment or treatment sequence as the replaced subject.

Table 7: Randomisation

Group	Randomization number	Replacement randomization number	Randomization ratio Verum:Placebo
S1	1101 to 1102	5101 to 5102	1:1
	1103 to 1108	5103 to 5108	5:1
S2	1201 to 1208	5201 to 5208	6:2
S3	1301 to 1308	5301 to 5308	6:2
S4	1401 to 1408	5401 to 5408	6:2
S5	1501 to 1508	5501 to 5508	6:2
S6	1601 to 1608	5601 to 5608	6:2
S7	1701 to 1708	5701 to 5708	6:2
S8	1801 to 1808	5801 to 5808	6:2
M1	2101 to 2108	6101 to 6108	6:2
M2	2201 to 2208	6201 to 6208	6:2
M3	2301 to 2308	6301 to 6308	6:2
M4	2401 to 2408	6401 to 6408	6:2
FI	3101 to 3112	7101 to 7112	NA
PD	4101 to 4120	8101 to 8120	NA

10.2. Blinding

This study is double-blind for the SAD, MAD and PD parts. The FI part is open label.

The unblinded pharmacist and his/her attendant will be the only personnel to have access to the randomisation list.

10.3. Emergency code-break procedure

In case of an emergency, when knowledge of the investigational product assignment is required for the medical management of an individual subject, the treatment for that subject may be unblinded. The investigator must notify the sponsor within 24 hours after determining that it is necessary to unblind the treatment assignment.

This documentation must include the name of the individual breaking the blind, the date on which the blind was broken, and a description of the event that led to the unblinding. The investigator must also indicate in source documents and in the CRF that the blind was broken and provide the date, time, and reason for breaking the blind. Any AE or serious AE (SAE) associated with breaking the blind must be recorded and reported as specified in this protocol.

Monitors will routinely check the integrity of the envelopes that are stored at the site. The envelopes will be collected from the site prior to study close-out and sent to the Sponsor to ensure that they were all intact.

11. PHARMACOKINETIC EVALUATION

11.1. Pharmacokinetic Criteria

The investigated pharmacokinetic criteria include:

In the SAD part:

PK Plasma parameters

The investigated plasma pharmacokinetic criteria include: the maximum observed plasma concentration (C_{max}), the time of occurrence of C_{max} (t_{max}), the terminal elimination rate constant (λz), the apparent terminal half-life ($t_{½}$), the area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}), the area under plasma concentration-time curve from hour 0 to infinity (AUC_{inf}), the apparent volume of distribution (Vz/F), the apparent total body clearance (CL/F), the last measurable plasma concentration (C_{last}) and the time to reach last measurable plasma concentration (t_{last}).

Additional parameters could be calculated if deemed necessary.

PK urine parameters

The investigated urine pharmacokinetic criteria include: the total amount excreted in urine (A_e), the percent of drug recovered in urine ($A_{e\ \%dose}$) and the apparent renal clearance (CL_r). Additional parameters could be calculated if deemed necessary.

In the MAD part:

PK Plasma parameters

- On D1: The investigated plasma pharmacokinetic criteria include: the maximum observed plasma concentration (C_{max}), the time of occurrence of C_{max} (t_{max}), the area under the plasma concentration-time curve over one dosing interval ($AUC_{0-\tau}$), the area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}), the last measurable plasma concentration (C_{last}) and the time to reach last measurable plasma concentration (t_{last}).

- On D10: The investigated plasma pharmacokinetic criteria include: the maximum observed plasma concentration (C_{max}), the time of occurrence of C_{max} (t_{max}), the area under the plasma concentration-time curve over one dosing interval ($AUC_{0-\tau}$), the area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}), the area under plasma concentration-time curve from hour 0 to infinity (AUC_{inf}), the elimination rate constant (λz), the apparent terminal half-life ($t_{½}$), the apparent volume of distribution (Vz/F), the apparent total body clearance (CL/F), the minimum observed plasma concentration (C_{min}), the average plasma concentration at steady state ($C_{average}$) calculated as $C_{average} = AUC_{0-\tau}/\tau$, the concentration at the end of a dosing interval before the next dose administration (C_{trough}), the peak-trough fluctuation (PTF%) calculated as PTF% = 100 x $[(C_{max}-C_{min})/C_{average}]$; the last measurable plasma concentration (C_{last}), the time to reach last measurable plasma concentration (t_{last}) and the accumulation ratio of C_{max} and $AUC_{0-\tau}$ (R_{acc} C_{max} and R_{acc} $AUC_{0-\tau}$) calculated as:
- R_{acc} $C_{max} = C_{max,\ Day\ 10} / C_{max,\ Day\ 1}$
- R_{acc} $AUC_{0-\tau} = AUC_{0-\tau,\ Day\ 10} / AUC_{0-\tau,\ Day\ 1}$

Additional parameters could be calculated if deemed necessary.

- *PK urine parameters*

The investigated urine pharmacokinetic criteria include: the total amount excreted in urine (A_e), the percent of drug recovered in urine ($A_{e\ \%\ dose}$) and the apparent renal clearance (CL_r).

Additional parameters could be calculated if deemed necessary.

In the FI part:

PK Plasma parameters

The investigated plasma pharmacokinetic criteria include: the maximum observed plasma concentration (C_{max}), the time of occurrence of C_{max} (t_{max}), the elimination rate constant (λz), the apparent terminal half-life ($t_{½}$), the area under plasma concentration-time curve from hour 0 to last sample with measurable plasma concentrations (AUC_{last}), the area under plasma concentration-time curve from hour 0 to infinity (AUC_{inf}), the apparent volume of distribution (Vz/F), the apparent total body clearance (CL/F),the last measurable plasma concentration (C_{last}) and the time to reach last measurable plasma concentration (t_{last}).

Additional parameters could be calculated if deemed necessary.

11.2. Pharmacokinetic Assessment Methods and Timing

Plasma

Venous blood samples (3 mL) for the determination of plasma concentrations of BIA 10-2474 and metabolites will be drawn by direct venipuncture or via an intravenous catheter into K3-EDTA tubes during the SAD, MAD and FI parts of the study.

It is important to avoid haemolysis during blood collection.

After collection, the blood samples will be immediately (no longer than 30 min) centrifuged, at approximately 1500 g for 10 minutes at 4°C. The resulting plasma will be separated into 2 equal aliquots of at least 250 µL each (series A & B) and transferred to polypropylene tubes, which will be labelled, frozen and stored at –80 °C until required for analysis. These plasma aliquots will be used for the determination of BIA 10-2474 and its metabolites.

The blood samples will be drawn at the time-points indicated in Flow chart 1 for the SAD part, Flow chart 2 for the FI part, Flow chart 3 for the MAD part, and Flow chart 4 for the PD part. When a sampling time is close to a meal time, the sample will be collected before the meal.

Urine

Urine will be collected in glass containers (adapted to the collected urine volume) at the designated time points in the SAD and MAD parts of the study (see Flow chart 1 for the SAD part, and Flow chart 3 for the MAD part), their volume will be recorded in the CRF.

Aliquots of 10 ml of the resulting urine will be transferred into 15 mL polypropylene tubes, and 30 µL of Tween 80 will be immediately added to the sample. It is important to rigorously vortex the sample and ensure that it is sufficiently homogeneous, and they will be frozen and stored at -80°C until required for analysis.

11.3. Sample handling and labelling

Sample tubes will be labelled with self-adhesive pre-printed labels able to withstand freezing temperatures.

All samples will be frozen in an upright position; they will be retained in the clinical freezers at a temperature of < -70°C nominal or on dry ice until transferred to the laboratory to be stored frozen at a temperature of -80°C nominal until assayed.

Each aliquot of plasma will be identified as follows:

- Line 1: Study 1BIAL35 / BIA-102474-101 (SAD, MAD or FI),
- Line 2: randomisation number,
- Line 3: theoretical day and time after drug administration (e.g., D1H24); for the FI part, the period will also be identified (e.g., P1D1H24),
- Line 4: PK assay (plasma),
- Line 5: time point number (e.g., 101 for plasma D1 pre-dose, 102 for plasma D1H0.5),
- Aliquot number.

Each aliquot of urine will be identified as follows:

- Line 1: 1BIAL35 / BIA-102474-101 (SAD or MAD),
- Line 2: randomisation number,
- Line 3: theoretical day interval of time after drug administration (e.g., D1[H24-H36]),
- Line 4: PK assay (urine),
- Line 5: time point number (e.g., 201 for urine D1 pre-dose, 202 for urine D1[H0-H4]),
- Aliquot number.

11.4. Sample shipment and storage

The samples will be shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.

Study samples for PK assays will be shipped to: SWISSBIO ANALYTICS AG, Sternenfeldstr. 14, CH-4127 Birsfelden, Switzerland.

11.5. Bioanalytical methods

Determination of plasma concentrations will be carried out in compliance with Good Laboratory Practices (GLP) utilising validated methods. Details of the methodologies used and the results obtained will be given in the bioanalytical reports.

12. PHARMACODYNAMIC ASSESSMENT

12.1. Pharmacodynamic criteria

Detailed descriptions of the PD parameters and of their calculation are provided below.

SAD, MAD and FI parts

FAAH activity

The investigated criteria include the maximum observed effect on FAAH activity (E_{max}); time to occurrence of E_{max} (t_{Emax}); area under the effect-time curve (AUEC), calculated by the trapezoidal rule.

FAAH activity will be expressed as the amount of deuterated ethanolamide (EA-d4) formed by the action of the FAAH in isolated leukocyte samples, on an anandamide substrate, in pmol per hour, per mL of blood (pmol/H/mL blood).

Besides, AEA and related FAAs like PEA, OEA and LEA will also be analysed.
Additional parameters could be calculated if deemed necessary.

The blood samples will be drawn at the time-points indicated in Flow chart 1 for the SAD part, Flow chart 2 for the FI part, Flow chart 3 for the MAD part, and Flow chart 4 for the PD part. When a sampling time is close to a meal time, the sample will be collected before the meal.

SAD part

Psychometric testing

The investigated criterion is the M-(marijuana) score.

PD part

Psychometric tests and scales

The investigated criteria include speed (expressed as the mean reaction time) for CRT, DV and for RVIP, accuracy for CRT (expressed as the number of correct answers), DV (expressed as the number of correct answers) and RVIP (expressed as the number of correctly detected targets), number of missed targets and number of false answers for DV and RVIP, immediate and delayed recall efficiencies for LMT (expressed as the number of words correctly recalled in immediate recall 1, mean number of words correctly recalled in all immediate recalls and number of words recalled in delayed recall), sleepiness score for SSS and tension-anxiety, depression-dejection, anger-hostility, vigour-activity, fatigue-inertia and confusion-bewilderment scores for POMS.

Additional parameters could be calculated if deemed necessary.

Pain model (cold pressor test)

The investigated criteria include pain and parameters derived from the pain VAS: the time endured with hand submerged in cold water (cold pressor tolerance-CP_{tol}), the maximum value observed (pain VAS_{max}), the total of values observed until the withdrawal from the bath (pain VAS_{total}), and the analog scale with replacement of missing values between time of withdrawal and 120 seconds by maximum value (100) (pain $AUC_{0\text{-}120s}$).
Additional parameters could be calculated if deemed necessary.

Cough challenge (capsaicin)

The investigated criteria include the concentrations of capsaicin causing five (C5) and two coughs (C2), the number of coughs during the first 15s after administration for each capsaicin concentration, the number of coughs from 15s after to 1 minute after administration for each capsaicin concentration.

Additional parameters could be calculated if deemed necessary.

IOP measurement

The investigated criterion is intraocular pressure (in mmHg).

Additional parameters could be calculated if deemed necessary.

Apomorphine challenge

The investigated criteria include emesis and parameters derived from the nausea VAS: the maximum degree of nausea (NS_{max}), the time of the maximum degree of nausea (NS t_{max}), the area under the degree of nausea versus time curve [NS $AUC_{(0\text{-}90\ mins)}$].

Additional parameters could be calculated if deemed necessary.

12.2. Pharmacodynamic Assessment Methods and Timing

12.2.1. FAAH activity

Venous blood samples (4 mL) for the determination of FAAH activity will be drawn by direct venipuncture or via an intravenous catheter into Li-Heparin tubes.

It is important to avoid haemolysis during blood collection.

When a sampling time is close to a meal time, the sample will be collected before the meal.

12.2.1.1. Sample handling and labelling

Sample tubes will be labelled with self-adhesive pre-printed labels able to withstand freezing temperatures.

Precisely 2.00 mL aliquots of human blood will be diluted in approximately 36.8 mL of cold (4°C) red cell lysis buffer. After 10 minutes of rock mixture at room temperature, samples will be centrifuged at 4°C for 10 minutes at 300 g. The supernatant will be carefully discarded and the pellet resuspended in 2.5 mL cold Phosphate-buffered saline (PBS) followed by centrifugation for 10 minutes at 800 g at 4°C. Again, the supernatant will be carefully discarded and the pellet immediately frozen and kept on dry ice until storage. Each aliquot will be stored at -80°C until required for analysis.

Each aliquot of leukocytes will be identified as follows:

- Line 1: 1BIAL35 / BIA-102474-101 (SAD, MAD or FI),
- Line 2: randomisation number,
- Line 3: theoretical day and time or interval of time after drug administration,
- Line 4: FAAH assay,
- Line 5: time point number (e.g., 301 for FAAH D1 predose, 302 for FAAH D1H0.5); for the FI part, the period will also be identified (e.g., P1D1H24),
- Aliquot number.

12.2.1.2. Sample shipment and storage

The samples will be shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.

Study samples for FAAH activity assays will be shipped to: SWISSBIO ANALYTICS AG, Sternenfeldstr. 14, CH-4127 Birsfelden, Switzerland.

12.2.1.3. Bioanalytical methods

FAAH assays will be carried out in compliance with GLP utilising validated methods. Details of the methodologies used and the results obtained will be given in the bioanalytical reports.

12.2.2. AEA and related FAAs

Venous blood samples (6 mL) for the determination of AEA and FAAs will be drawn by direct venipuncture or via an intravenous catheter into K3-EDTA tubes during the SAD, MAD and FI parts of the study.

It is important to avoid haemolysis during blood collection.

After collection, the blood samples will be immediately (no longer than 30 min) centrifuged, at approximately 1500 g for 10 minutes at 4°C. The resulting plasma will be separated into 2 equal aliquots of at least 1.5 mL each (series A & B) and transferred to polypropylene tubes, which will be labelled, frozen and stored at –80 °C until required for analysis. These plasma aliquots will be used for the determination of AEA and related FAAs.

12.2.2.1. Sample handling and labelling

Sample tubes will be labelled with self-adhesive pre-printed labels able to withstand freezing temperatures.

All samples will be frozen in an upright position; they will be retained in the clinical freezers at a temperature of < -70°C nominal or on dry ice until transferred to the laboratory to be stored frozen at a temperature of -80°C nominal until assayed.

Each aliquot of plasma will be identified as follows:

- Line 1: Study 1BIAL35 / BIA- 10-2474-101,
- Line 2: randomisation number,
- Line 3: theoretical day and time after drug administration (e.g., D1H24),
- Line 4: AEA & FAAs assay (plasma),
- Line 5: time point number (e.g., 101 for plasma D1 pre-dose, 102 for plasma D1H0.5),
- Aliquot number.

12.2.1.2. Sample shipment and storage

The samples will be shipped in a container filled with enough dry ice to ensure that the samples are kept frozen.
Study samples for AEA and related FAAs will be shipped to: NUVISAN GmbH, Wegenerstrasse 13, 89231 Neu-Ulm, Germany.

12.2.1.3. Bioanalytical methods

AEA and FAAs assays will be carried out in compliance with GLP utilising validated methods. Details of the methodologies used and the results obtained will be given in the bioanalytical reports.

12.2.3. Cognitive testing

In the SAD part:

M-scale will be used at T0 before dosing and at 3h post dose. A familiarization session will be done on D-1.

In the PD part:

Several psychometric tests and scales will be used, including CRT, DV, RVIP, LMT, SSS, and POMS.

The baseline will be established prior to dosing on D-1. The tests will then be administered at the predicted t_{max} on D1, D5 and D9.

As performance on CRT, DV, RVIP tests shows a learning component, a training session will be organised on D-1 morning so that volunteers can reach their maximal performance prior to the study. These tests will therefore be done at least 4 times.

The tests without learning effect (LMT and subjective evaluations) will be done only once to familiarise the subjects with the procedure.

All psychometric tests and scales will be computerized; this computer-assisted testing takes approximately 30 minutes and allows rapid electronic transfer into the study database.

12.2.2.1. M-scale

The marijuana (M) scale is a self-reporting instrument specifically designed to measure cannabis intoxication. It is a subscale (12 true-false statements) of the Addiction Research Center Inventory (ARCI) which was developed by Chait et al. (1985) and has been demonstrated to be sensitive to the effects of smoked marijuana [7].

12.2.2.2. Choice reaction time (CRT)

This test evaluates the speed at which a subject is able to respond to a complex visual stimulus. The complex stimulus consists of the words YES or NO, which will appear randomly at the centre of the computer screen. The subject is asked to press the green button (! button) of the keyboard as quickly as possible if the word YES appears on the screen, or to press the red button (W button) as quickly as possible if the word NO appears on the screen. Fifty (50) targets (25 YES and 25 NO) will be presented to the subject. This test takes approximately 3 minutes. Performance is assessed by the speed, expressed as the mean reaction time in msec, and the accuracy, expressed as the number of correct answers.

12.2.2.3. Digit vigilance (DV)

This test assesses sustained attention. A target digit is randomly selected and constantly displayed to the right of the screen. A series of 500 digits is then presented in the centre of the screen at the rate of 150 per minute and the subject is required to press the space bar of the keyboard as quickly as possible every time the digit in the series matches the target digit.
There are 50 targets in the series. The test takes approximately 4 minutes. The performance is the speed, expressed as the mean reaction time in msec, and the accuracy, expressed as the number of correct answers. In addition, missed targets and false answers are recorded.

12.2.2.4. Rapid visual information processing (RVIP)

This test assesses the effects of drugs on sustained attention and working memory. A series of 1000 digits is presented in the centre of a computer screen at a rate of 100 digits every 70 sec. The subject is instructed to press the space bar of the keyboard as quickly as possible when they detect sequences of three consecutive odd or three consecutive even digits. On average, 80 of these sequences (40 odd sequences and 40 even sequences) are presented over 10 minutes. Any two sequences are separated by a minimum of 5 and a maximum of 30 digits. After the appearance of the third digit of an experimental target, 1500 msec are allowed for a correct response to be made. Responses obtained at any other time are counted as errors. The performance is the speed, expressed as the mean reaction time in msec, and the accuracy, expressed as the number of correctly detected targets. In addition, missed targets and false answers are recorded.

12.2.2.5. Learning memory task (LMT)

This test involves learning a list of 15 words which are presented to the subject on a computer screen at a rate of one word every 900 msec. It assesses short- and long-term memory. After the presentation of the 15 words, the subject is asked to recall freely as many words as possible in any order in 60 seconds. This process will then be repeated until 4 trials have elapsed, with the 15 words presented in a different order at each trial. Delayed free recall is recorded about 30 minutes later, when the subject is then given 60 seconds to recall freely as many words as he can remember. Equivalent lists are constructed using commonly used words of 2 syllables, selected according to their degree of concreteness, imagery and meaningfulness. At each time and day, as requested in the protocol, a different list of words (equivalent) will be presented to the subject. The scores are the number of words recalled after the first immediate recall (immediate free recall 1), and the mean number of words correctly recalled in all immediate recalls (mean immediate free recall) and in the delayed free recall.

12.2.2.6. Stanford Sleepiness Scale (SSS)

The Stanford Sleepiness Scale is used to provide a subjective assessment of the subject's general level of daytime sleepiness. The SSS consists of a seven-point scale of equal intervals varying from 1 ("feeling active and vital; alert; wide awake ") to 7 ("almost in reverie; sleep onset soon, lost in struggle to remain awake"). The score is expressed between 1 and 7.
It must be taken into account that most people have two peak times of alertness daily, at about 9 a.m. and 9 p.m. Alertness wanes to its lowest point at around 3 p.m.; after that it begins to build again.

12.2.2.7. Profile of Mood States (POMS)

The Profile Mood Scale consists of 65 adjectives describing various mood feelings. A computerized version of this paper-pencil questionnaire will be used in this study. Each adjective will appear one by one on the screen and the subject is asked to describe how these adjectives reflect his mood at the time he is completing the questionnaire, rating each description on a 5 point scale of increasing agreement: from "not at all" to "extremely". Six scores are classically derived from the questionnaire: tension – anxiety (TA), depression – dejection (DD), anger – hostility (AH), vigor – activity (VA), fatigue – inertia (FI), confusion – bewilderment (CB).

12.2.4. Pain model: Cold Pressor Test

In the PD part, the cold pressor test will be used in order to induce pain and to verify a potential antalgic effect of BIA 10-2474. This test will be done during the screening visit in order to verify that the study subjects are all responsive to the predefined cold pressor tolerance test. This test will then be used as a pain model on Day 6 at t_{max}.

The cold pressor test will be performed by having the subjects submerge their dominant hand and wrist in circulating cold water and hold it there as long as they are able, up to 120 seconds. Endurance time (time to remove hand) will be recorded. The refrigerated circulator is connected to a container with a water temperature of 3.0°C and flow rate of 22 L/min; Subjects will rate their pain intensity every 10 seconds until the hand is withdrawn from the bath on a pain VAS. This latter is a 100 mm VAS ranging from "no pain at all" to "the maximum pain that you can imagine" and will be used at all-time points.

Cold pressor tolerance (CP_{tol}) is assessed by the time endured with the hand submerged in cold water and is considered as the main outcome.
In addition to CP_{tol}, the pain intensity will be analysed as the maximum value observed (VAS_{max}), the total of values observed until withdrawal from the bath (VAS_{total}), and $AUC_{0\text{-}120s}$, which is the analogue scale with replacement of missing values between time of withdrawal and 120 seconds by the maximum value (100).

12.2.5. Antitussive model: Cough challenge (capsaicin)

In the PD part, capsaicin will be used in order to experimentally induce cough and to assess a potential antitussive effect of BIA 10-2474. This test will be done during the screening visit in order to verify that the study subjects are all responsive to the cough provocation agent (capsaicin) at the concentration tested at screening. This cough challenge will then be used as an antitussive model on Day 7 at t_{max}.

According to the European Respiratory Society (ERS) guidelines [8], the methodology for the performance of inhalation cough challenge is standardized in order to facilitate universal interpretation and comparison of data.
For this reason, the cough challenge test will use the single-breath concentration–response method using a flow-limited dosimeter, and both C2 and C5 will be recorded [i.e., the concentrations of capsaicin causing two (C2) and five coughs (C5)].
Capsaicin, the non-acid tussive agent most commonly used to experimentally induce cough in humans, was first described in 1984 and is well known to induce cough in a dose-dependent and reproducible manner.
Studies in healthy volunteers are almost invariably performed using cough challenge methodologies. Subject selection includes a screening visit, during which the challenge is performed. There is a strong argument for excluding subjects who show cough responses only at high challenge concentrations. In these subjects, it is difficult to demonstrate cough suppression because they are already approaching the maximum tolerable dose, and nonspecific effects, such as a burning sensation from capsaicin, mask any active effect.

Materials:
In order to control inspiratory flow rate, the device used will be a compressed air-driven nebulizer controlled by a dosimeter, modified by the addition of an inspiratory flow regulator valve.
The valve limits inspiratory flow rate to 0.5 L/sec regardless of excessive inspiratory force, thereby guaranteeing a consistent and reproducible inspiratory effort with each breath. Thus, with appropriate instructions to inhale with sufficient force, all subjects achieve an identical inspiratory flow rate during each inhalation of aerosol.
Using these devices, the exact output (in mL/min) of the nebulizer is determined and the modulation of the duration of aerosol delivery permits the determination of aerosol output per inhalation. For example, a nebuliser with an output of 1.007 mL/min, programmed to deliver aerosol for 1.2 s, provides 0.02 mL/breath.

Measurements:
Capsaicin cough challenge will be performed by inhalation of incrementally doubling concentrations of capsaicin (0.9765-500 µmol/L). The dosimeter will deliver a puff of 20 µL per administration. Up to 10 concentrations of capsaicin could be tested by the volunteer to determine the concentration causing two and five coughs at screening (=baseline) and on Day 7 of each period.

Determination of tussive response to cough challenge:
When employing the single-breath method of capsaicin administration, the tussive response to each dose of aerosol is immediate and brief.
Subjects undergoing cough challenge should be specifically instructed not to attempt to suppress any coughs and not to talk immediately after inhalation of the tussive agent, since this may potentially suppress cough. It is recommended, for example, to give the following instruction to subjects: ''allow yourself to cough if you need to and as much as you need to''. Subjects should not be told that the induction of a specific number of coughs is the end-point of the study.
Therefore, only coughs occurring within 15s of capsaicin delivery should be counted. Coughs that occur beyond this time period may not be capsaicin-induced, but will nevertheless be counted.

Interpretation of cough challenge data:
For each test, the concentrations of capsaicin causing two (C2) and five coughs (C5) will be reported. The C2 and C5 can be obtained by determining the first administered concentration that results in two or more and five or more coughs; the number of coughs during the first 15s after administration for each capsaicin concentration. The number of coughs from 15s after to 1 minute after administration for each capsaicin concentration will also be counted.

12.2.6. Intraocular pressure measurement

In the PD part, on Day 8 at t_{max}, IOP will be measured by an ophthalmologist using an applanation tonometer.

12.2.7. Apomorphine challenge

In the PD part, apomorphine will be used in order to experimentally induce nausea and/or emesis and to assess a potential anti-emetic effect of BIA 10-2474. This apomorphine challenge will be used as an anti-emetic model on Day 10 at t_{max}.

Each subject will receive a single 50 µg/kg subcutaneous injection of apomorphine. Apomorphine will be administered around the t_{max} of BIA 10-2474, as determined in the SAD part/MAD part.

The capacity of BIA 10-2474 to inhibit apomorphine-induced nausea and/or emesis will be evaluated by recording the occurrence of vomiting, the total number of retches and vomits, and the degree of nausea after having administered 50 µg/kg of apomorphine. Nausea, retches and/or vomiting are expected to occur within 10 minutes on average after the injection of apomorphine, with duration of approximately 5 to 45 minutes.

The emesis will be evaluated over 90 minutes after apomorphine injection as follows:

- Occurrence of vomiting : Yes/No
- Total number of vomits and retches
- Degree of nausea using a 100 mm VAS ranging from 0 (no nausea) to 100 (severe nausea). The volunteers will be asked to record the degree of nausea using a 100 mm VAS just before the apomorphine injection and at 10, 20, 30, 40, 50, 60, 70, 80, and 90 minutes after injection. During the emesis challenge test, subjects will be in separate rooms.

The following parameters will be derived and analysed: Maximum degree of nausea (NS_{max}), time of the maximum degree of nausea (NS t_{max}), area under the degree of nausea versus time curve [NS $AUC_{(0\text{-}90\ mins)}$].

13. SAFETY ASSESSMENT

13.1. Physical Examination

In all study parts, physical examination will be performed at screening, at admission visit, at discharge and at follow-up visit.

Body weight and height are measured at screening visit in order to calculate the BMI (inclusion criteria #2).

Physical examination can also be performed throughout the study on medical indication at the discretion of the medical investigator.

The physical examinations will be performed by the Investigator or his representatives.

13.2. Neurological Examination

A complete neurological examination will be performed at screening in the four study parts, and at discharge in the PD part.

13.3. Vital Signs

13.3.1. Parameters

- Measured parameters: Supine and standing systolic blood pressure (SBP), diastolic blood pressure (DBP) and pulse rate, and body temperature.

13.3.2. Method of assessment

Qualified staff members will perform BP measurements. If possible, BP measurements will be taken from the same arm (opposite the arm that is used for blood sample collection) by an automated BP monitor using the oscillometric method (e.g., Dinamap®). In the case of an out-of-range value, measurements will be repeated immediately to confirm the change. Normal ranges for vital signs parameters used will be according to Biotrial standard operating manual (SOP).

Orthostatic vital signs (supine and standing BP and pulse) will be obtained:

- In the SAD part: at screening, D-2, D-1, D1, D2, D3, D4 at discharge, and at the Follow-up Visit.
- In the FI part: at screening, and for each period at D-1, D-2, D1, D2, D4 at discharge, and at the Follow-up Visit.
- In the MAD part: at screening, D-2, D-1, D1, D2, D10, D11, D12, D13 at discharge, and at the Follow-up visit.
- In the PD part: at screening, and for each period at D-1, D1, D2, D9, D10, D11, D12 at discharge, and at the Follow-up Visit.

Supine BP and pulse will be measured after the subject has been supine for at least 5 minutes. Standing BP and pulse will be measured after the subject has been standing for 2–3 minutes.

On D-1, vital signs will be obtained in triplicate at approximately 15 to 30 minute intervals. The subjects will be made to lie supine for at least 5 minutes before supine BP measurement and to stand for 2–3 minutes before the standing measurement. This process will be repeated 3 times in the same conditions each time. The average of the triplicate vital sign measurements will serve as baseline.

Tympanic body temperature will be measured:

- In the SAD part: at screening, D-2, D4/discharge, and at the Follow-up Visit.
- In the FI part: at screening, and for each period at D-2, D4/discharge, and at the Follow-up Visit.
- In the MAD part: at screening, D-2, D13/discharge, and at the Follow-up visit.
- In the PD part: at screening, and for each period at D-1, D12/discharge, and at the Follow-up Visit.

When vital signs are scheduled at the same time as blood draws, the blood draws will be obtained at the scheduled time point, and the vital signs will be obtained as close to the scheduled blood draw as possible. The timing of the assessments is summarised in Flow chart 1 for the SAD part, Flow chart 2 for the FI part, Flow chart 3 for the MAD part, and Flow chart 4 for the PD part.

13.4. Standard 12-lead ECG

13.4.1. Parameters

- Measured parameters: HR, PR, QRS duration, QRS axis, QT;
- Derived parameters: two corrections of the QT interval will be investigated: Fridericia's correction (QTcF) and Bazett's correction (QTcB).
- Observations and comments on the quality of trace, on normality or abnormality.

13.4.2. Method of assessment

ECG recordings will be made using a Cardionics® ECG system.

12-lead ECG recordings will be performed:

- In the SAD part: at screening, D-2, D-1, D1, D2, D3, D4 at discharge, and at the Follow-up Visit.
- In the FI part: at screening, and for each period at D-1, D-2, D1, D2, D4 at discharge, and at the Follow-up Visit.
- In the MAD part: at screening, D-2, D-1, D1, D2, D10, D11, D12, D13 at discharge, and at the Follow-up visit.
- In the PD part: at screening, and for each period at D-1, D1, D2, D9, D10, D11, D12 at discharge, and at the Follow-up Visit.

The timing of the assessments summarised in Flow chart 1 for the SAD part, Flow chart 2 for the FI part, Flow chart 3 for the MAD part, and Flow chart 4 for the PD part. Triplicate ECGs will be obtained on D-1 at approximately 15 to 30 minute intervals. The average of the triplicate ECGs will serve as baseline.

The measurements will consist of 12-lead digital ECGs: D1, D2, D3, aVr, aVL, aVf, V1 to V6. Normal ranges for ECG parameters will be according to Biotrial SOP.

Subjects must rest in the supine position for at least 5 minutes before the ECG recording is started. The ECG may be recorded during the period of rest required before the measurements of supine BP and pulse. A qualified physician will review the ECGs promptly and any clinically important finding will be recorded on the appropriate CRF. The investigator is responsible for providing the interpretation of all ECGs.

13.5. Telemetry

13.5.1. Parameters

Cardiac telemetry is continuous monitoring of a patient's heart rate and rhythm.

13.5.2. Method of assessment

Telemetry will be used:

- In the SAD part: from pre-dose to 24h post-dose on D1 only
- In the MAD part: from pre-dose to 24h post-dose on D1 and from pre-dose to 24h post-dose on D10.

Telemetry system allows a remote monitoring of the cardiac rhythm of the subjects. This is a wireless device allowing subjects to move around freely in the clinical unit. A centralised, continuous and automatic evaluation of the ECG signal is carried out. Alerts are triggered as soon as an abnormal event occurs (arrhythmias). These alerts are reviewed by the investigator and considered for safety management of the subjects.

The system used at Biotrial is Cardiovision by Cardionics.

13.6. Laboratory safety parameters

13.6.1. Serology, Drug Screen, Pregnancy Tests, Hormonology, and Risk Factors

Blood tests will be carried out to test for the presence of HIV-1 and 2 antibodies, HCV antibodies and HBs Ag.

Urinary screening will be carried out to test for amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates.

Alcohol breath tests will be performed.

Serology will be carried out only at screening (for the four study parts).

Drug and alcohol screens will be carried out at screening and at each admission (D-2 for SAD, FI and MAD parts, and D-1 for PD part).

Female subjects of childbearing potential will be tested for pregnancy, in serum at screening and at the Follow-up Visit, and in urine at each admission.

FSH levels will be measured in post-menopausal female subjects only if less than12 months post last menstrual period.

13.6.2. Laboratory Safety

13.6.2.1. Blood safety analysis

The following parameters will be determined:

- **Haematology:**

Red blood cell count, haemoglobin, haematocrit, white blood cell count with differential (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelets count.

- **Coagulation:**

Activated partial thromboplastin time (APTT), international normalized ratio (INR).

- **Biochemistry:**

Sodium, potassium, calcium, chloride, total and conjugated bilirubin, alanine aminotransferase (AST), aspartate aminotransferase (ALT), gamma-glutamyl transferase (GGT), alkaline phosphatases, total protein, albumin, urea, uric acid, bicarbonate, creatine phosphokinase (CPK), creatinine, glycaemia, lactate dehydrogenase (LDH).

13.6.2.2. Urinalysis parameters

Determination of pH, protein, glucose, leukocytes, nitrites, ketones, and blood will be performed.

13.6.2.3. Method of assessment

Clinical laboratory assessments and samples of urine are scheduled:

- In the SAD part: at screening, D-2, at discharge, and at the Follow-up Visit.
- In the FI part: at screening, and for each period at D-2 and at discharge, and at the Follow-up Visit.

- In the MAD part: at screening, D-2, D5 (predose), at discharge, and at the Follow-up Visit.
- In the PD part: at screening, and for each period at D-1, at discharge, and at the Follow-up Visit.

All clinically significant abnormal laboratory test values identified after IMP administration will be repeated until the values return to normal or baseline. If laboratory values do not return to normal or baseline within a reasonable period, the aetiology should be identified and the Sponsor notified.

The Safety urinalysis will involve semi-quantitative analysis (dipsticks): pH, protein, glucose, leukocytes, nitrites, ketones, blood.

13.6.2.4. Laboratory safety determinations

Laboratory tests will be performed for Biotrial Rennes by the Centre de Lutte contre le Cancer Eugène Marquis, Département de Biologie Clinique, Rue de la Bataille de Flandres-Dunkerque – CS44229 - 35062 Rennes Cedex.

13.7. Adverse Events and Treatment Emergence

13.7.1. Definitions

- **Adverse Event**

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with the treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE includes, but is not limited to, the following:

- Any clinically significant event, or worsening of a pre-existing condition,
- An AE occurring from overdose of an IMP, whether accidental or intentional. Overdose is a dose greater than specified in the protocol,
- An AE occurring from abuse (e.g., use for nonclinical reasons) of an IMP,
- An AE that has been associated with the discontinuation of the use of an IMP.

Laboratory/ECG/vital signs abnormalities should not be documented as AEs unless they are considered clinically relevant, require treatment, fulfil any SAE criterion, or cause the subjects to change the study schedule.

In the case of laboratory/ECG abnormalities that are a sign of a medical condition, the condition should be reported as an AE, and not the sign.

Events occurring in subjects in the course of a clinical study during treatment-free periods or on treatment with placebo or a comparative medicine are also to be considered AEs.

- **Treatment Emergent Adverse Event and Repetition of Adverse Event**

A treatment-emergent AE is any AE that occurs after dosing, or that was present prior to dosing but is exacerbated after dosing.

An AE which occurs more than once in the same subject is a repetition and will only be counted once. A clinically relevant worsening of an AE (e.g., relevant change in severity, seriousness) must result in a new entry. The original entry remains unresolved and is given an end date reflecting the date of the worsening and a comment must be entered stating that the AE is continuing with a changed severity/seriousness (e.g., "continues as *event name* with *onset date* and *new severity/seriousness*"). The onset date of the new entry is also the date of worsening.

13.7.2. Serious adverse events or Serious Adverse Drug Reactions

- **General definitions:**

A SAE or Serious Adverse Drug Reaction (ADR) is any untoward medical occurrence that at any dose:

1. Results in death
2. Is life-threatening (see below)
3. Requires inpatient hospitalisation or prolongation of an existing hospitalisation (see below)
4. Results in a persistent or significant disability or incapacity (see below)
5. Is a congenital anomaly or birth defect

Additionally, important medical events may be considered SAEs when, based on appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or drug abuse.

Life-threatening refers to immediate risk of death as the event occurred. A life-threatening experience does not include an experience that, had it occurred in a more severe form, might have caused death, but that, as it actually occurred, did create an immediate risk of death. For example, hepatitis that resolved without evidence of hepatic failure would not be considered life-threatening, even though hepatitis of a more severe nature can be fatal. Similarly, an allergic reaction resulting in angioedema of the face would not be life-threatening, even though angioedema of the larynx, allergic bronchospasm, or anaphylaxis can be fatal.

Hospitalisation is official admission to a hospital. Hospitalisation or prolongation of a hospitalisation constitutes criteria for an AE to be serious; however, it is not itself considered a SAE. In the absence of an AE, a hospitalisation or prolongation of a hospitalisation should not be reported as an SAE. This is the case in the following situations:

- The hospitalisation or prolongation of hospitalisation is needed for a procedure required by the protocol.
- The hospitalisation or prolongation of hospitalisation is part of a routine procedure followed by the centre (e.g., stent removal after surgery). This should be recorded in the study file.

In addition, hospitalisation for a pre-existing condition that has not worsened does not constitute an SAE.

Disability is defined as a substantial disruption in a person's ability to conduct normal life functions.

If there is any doubt about whether the information constitutes an SAE, the information is treated as an SAE.

Other Reportable Information

Certain information, although not considered an SAE, must be recorded, reported, and followed up as indicated for an SAE. This includes:

- Overdose of an investigational product as specified in this protocol with or without an AE.
- Inadvertent or accidental exposure with or without an AE.

Expected and unexpected adverse events:

As this is a first-in-man study, no AEs are defined as expected.

13.7.3. Severity

The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient's daily activities.

Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

13.7.4. Causal relationship with trial medication

The relationship of an AE to the IMP is a clinical decision by the investigator based on all available information at the time of the completion of the CRF and is graded as follows:

1. **Not related:** a reaction for which sufficient information exists to indicate that the aetiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is clearly related to other factors such as the subject's clinical state, therapeutic intervention or concomitant therapy.

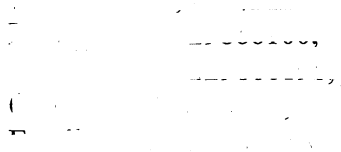
2. **Unlikely:** a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.
3. **Possible:** a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.
4. **Probable:** a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfil this definition.
5. **Definite:** a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

13.7.5. Documentation and Treatment of Adverse Events by the Investigator

All AEs, including SAEs, occurring within the period of observation for the clinical study must be recorded.

The **period of observation** for the collection of AEs extends from the time when the subject gives Informed Consent until 10 to 14 days after the end of the treatment period. For all subjects, this period will be extended to follow-up on all on-going AEs after the end of the treatment period until all AEs are finally resolved or it is medically justifiable to stop further follow-up (e.g., a chronic condition has been reached).

There is no time limit on the collection of SAEs that are considered related to study drug. If the Investigator detects an SAE in a study subject after the end of the period of observation, and considers the event possibly related to prior study treatment or procedures, he must contact Bial's Head of Pharmacovigilance and Drug Safety Management to determine how the SAE should be documented and reported.
Bial's Head of Pharmacovigilance and Drug Safety Manager Contact details are:



The AEs must be documented as soon and as completely as possible on the "Adverse Events" pages in the CRF. Follow-up information must be entered as soon as available.

The following will also be specified:

- Other actions (none, medication required, tests required, hospitalisation required or prolonged, treatment unblinded, other-specify)

- Outcome and date of outcome according to the following definitions:
    - Recovered/resolved
    - Recovering/resolving
    - Not recovered/not resolved
    - Recovered with sequelae/resolved with sequelae
    - Fatal
    - Unknown
- Seriousness: yes or no (criteria for SAE see above)

Adverse events which occur during the study should be treated by established standards of care that will protect the life and health of the subjects. If such treatment constitutes a deviation from the protocol, the subjects should be withdrawn from the study and the reason must be documented in the CRF.

13.7.6. Reporting of Serious Adverse Events

All serious adverse events including other information reportable as SAEs and follow-up information must be reported to the Sponsor within 24h of awareness by faxing a completed serious adverse event form and confirming by phone or e-mail that the fax was received.

Suspected Unexpected Serious Adverse Reactions (SUSARs) are subject to expedited reporting. Biotrial, on behalf of the Sponsor, will ensure all required information is entered in an initial report submitted to the regulatory agency (ANSM) and Ethics Committee as soon as possible, but no later than 7 calendar days after first knowledge, followed by as complete a report as possible within 8 additional calendar days in case of fatal or life-threatening SUSARs, or 15 calendar days for other SUSARs that are not fatal or life-threatening.

13.7.7. Pregnancy

All initial reports of pregnancy in female subjects or partners of male subjects must be reported to the Sponsor by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form. Abnormal pregnancy outcomes (e.g., spontaneous abortion, fetal death, stillbirth, congenital anomalies, and ectopic pregnancy) are considered SAEs and must be reported using the Serious Adverse Event Form. Any subject who becomes pregnant during the study must be promptly withdrawn from the study and discontinue further study treatment.

Because the effect of the study drug on sperm is unknown, pregnancies in partners of male subjects included in the study will be reported by the study-site personnel within 24 hours of their knowledge of the event using the appropriate pregnancy notification form.

Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be required.

14. DATA MANAGEMENT AND STATISTICS

14.1. Data entry and management

14.1.1. Data collection

All the results from evaluations conducted during the study will be recorded in an appropriate CRF for each subject.

Details of the procedure followed for filling in the CRF and making corrections are provided in Section 16.

All supportive documentation submitted to the Sponsor in addition to the CRF, such as laboratory or hospitalisation records, must be clearly identified with the study or protocol number, the study subject's number and initials; any personal information, including the study subject's name, must be removed or rendered illegible to preserve individual confidentiality.

All the documents must be archived for a minimum of 15 years.

14.1.2. Data coding:

Biotrial will ensure coding for subject AEs, concomitants medications and previous illnesses in compliance with the Medical Dictionary for regulatory activities (MedDRA) and the World Health Organisation Drug Dictionary (WHO-DD), using the most recent versions of MedDRA and WHO-DD available at the time of database set-up.

14.1.3. Data transfer

Biotrial will prepare the data validation document.

Biotrial will be responsible for data entry and their validation (according to the sponsor requirements). Data will be subject to double data entry.

Biotrial will organise a Data Review meeting before locking the database.

14.2. Statistical considerations

14.2.1. Sample size

Due to the exploratory nature of this investigation, there is no formal statistical hypothesis testing. The sample size is based on empirical considerations and on the literature [5; 6].

14.2.2. Statistical methods

Safety data will be analysed by Biostatistics Unit of BIOTRIAL using SAS® software version 9.3 or higher release (SAS Institute Inc., Cary, NC, USA).

Pharmacokinetic and pharmacodynamic data will be analysed by Biotrial using Phoenix® and SAS® software Version 9.3 or higher release (SAS Institute Inc. Cary NC USA).

No PK/PD correlation analysis will be done.

Descriptive statistics will be supplied according to the nature of the criteria:

- Quantitative variable: sample size, arithmetic mean, standard deviation (SD), median, minimum and maximum (with geometric mean and coefficient of variation (CV) for PK)
- Qualitative variable: sample size, absolute and relative frequencies per class

The analysis will be performed for each part and the tables, figures and listings will be edited for each part separately.

Data will be organised by treatment group with all the placebo-treated subjects pooled in a single group for the SAD and MAD parts.

14.2.3. Description of data sets

14.2.3.1. Definition of data sets

The following analysis data sets will be defined for each part separately.

For the SAD, MAD, FI and PD parts:

The Included set will be defined as all included subjects.

The Randomised set will be defined as all randomised subjects.

The Safety set will be defined as all included subjects having taken at least one dose of IMP.

For the SAD, MAD and FI parts:

The PK set will be defined as all randomised subjects having taken at least one dose of IMP without protocol deviation affecting PK evaluation and with available PK data.

The PD set will be defined as all randomised subjects having taken at least one dose of IMP without protocol deviations affecting PD evaluation and with available data for FAAH activity.

For the PD part:

The PD set will be defined as all randomised subjects having taken at least one dose of IMP without protocol deviations affecting PD evaluation and with available data for PD parameters.

The analysis sets will be validated during the data review meeting.

14.2.3.2. Description of the sets

A summary table with the description of the number of included subjects, number of randomised subjects, number of subjects who completed study, number of subjects who discontinued classified by reason of withdrawal, number of subjects in each analysis data set will be prepared. Corresponding individual listings will be prepared.

Listings with end of study status and visit dates will also be carried out by subject.

14.2.4. Demographic and baseline characteristics

The following analysis will be performed on the Randomised set.

The subjects' demographic characteristics and baseline characteristics will be summarized by treatment group/sequence and overall and listed by subject.

Tables by treatment group/sequence and overall with the number of subjects having at least one medical or surgical history and the corresponding listing by subject will be prepared.

Tables by treatment group/sequence and overall with the number of subjects having at least one previous treatment and the corresponding listing by subject will be prepared. The same table and listing will be prepared for concomitant treatments.

Details of drug dosing will be listed by subject.

14.2.5. Protocol deviations

A summary table of protocol deviations by treatment group/sequence and overall and the corresponding listing by subject will be generated.

14.2.6. Pharmacokinetic analysis

Single and multiple-dose PK parameters will be derived from the plasma concentration time and urinary excretion data.

A compartmental or non-compartmental PK method, as appropriate, will be used to analyse the plasma and urine concentrations of BIA 10-2474 and its metabolites.

Individual and mean plasma and urine concentrations at each sampling time point will be presented by listings and descriptive summary statistics for each dose group/food condition. Time profile plots of each dose group/food condition, arithmetic and logarithmic means (±SEM) at each time measurement will be generated for urine and plasma data.
All PK parameters will be presented by individual listings and summary statistics for each dose group/food condition. Box-plots will be performed by dose group/food condition for PK parameters.

For the SAD and MAD parts, the dose proportionality of PK plasma parameters C_{max} and AUC will be evaluated using the Power model. The PK parameters will be log-transformed prior to this statistical analysis in order to use the linear mixed effects model that includes Dose as a fixed effect. Dose proportionality will be analysed by using the estimated slope parameter of the linear model and the associated 90% confidence interval that measures the degree of non-linear proportionality.

For the MAD part, the steady state will be also studied for each dose group using a one-way analysis of variance on factor Day after logarithmic transformation on plasma concentrations.

For the evaluation of the food interaction, an analysis of variance model appropriate for a 2-period, cross-over design with fixed terms for sequence, period, and treatment (i.e., food condition) and a random term for subject within sequence will be used to investigate the food interaction on the C_{max} and AUC. Following log-transformation of the data, the 90% confidence intervals (90% CI) for the geometric mean ratio (GMR) between the food conditions will be calculated.
Moreover, t_{max} will be compared between the food conditions using a Wilcoxon signed ranks test.

Additional details for statistical consideration for PK analysis will be defined in the Statistical Analysis Plan (SAP).

14.2.7. Pharmacodynamic analysis

14.2.7.1. Criteria

The pharmacodynamics criteria are specified in Sections 4.5.3 and 12.1.

14.2.7.2. Statistical methodology

Analysis of pharmacodynamics parameters will be performed on the PD set.

Additional details for statistical consideration for PD analysis will be defined in the SAP.
All values and calculated parameters will be listed.

SAD\MAD\FI parts

FAAH activity, AEA and related FAAs in SAD\MAD\FI parts:

Raw data and changes from baseline* (percentage of inhibition for FAAH) will be described by treatment group\food condition and measurement time.
Time profile plots of arithmetic means (+SEM) at each measurement time will be generated by treatment group\food condition.

E_{max}, T_{Emax} and AUEC (on percentage of inhibition for FAAH) will be summarised by treatment group\food condition and Day (if applicable) using descriptive statistics.

* Baseline will be defined as Day -1 time-matched values.

For the SAD and MAD parts, an analysis of variance will be performed using log-transformed data for E_{max} and AUEC with treatment group as fixed effect. Geometric mean ratios (GMR) (with associated confidence intervals) between each treatment group and placebo group will be calculated.

Moreover, for the SAD and MAD parts, t_{Emax} will be compared between each treatment group and placebo group using a Mann-Withney Wilcoxon test.

To characterize the offset of the effect, the time needed to decrease by 50% the maximal effect will be investigated for ethanolamides (AEA and related FAA).

No adjustment will be performed as all these analyses will be exploratory.
Box whisker plots will be generated for the comparison of E_{max} and AUEC parameters between treatment groups\food condition.

For FAAH activity and active treatment groups, plots of individual data between plasma concentrations on X axis and percentage of inhibition (and raw data) on Y axis will be produced separately for SAD and MAD (and also by day for MAD).

Relationships between inhibition of FAAH and ethanolamides (AEA and related FAA) will be assessed in an exploratory manner.

Psychometric scale in the SAD part:

Raw data and changes from baseline (D1 predose) will be described by treatment group.
Total score will be analyzed using an analysis of variance with treatment group as fixed effect on the changes from baseline. Estimate of the difference (with associated CI) between each treatment group and placebo group will be calculated. No adjustment will be performed as this analysis will be exploratory.

PD part

Psychometric tests and scales
All the parameters will be described by treatment group and measurement time.

For all parameters except the number of missed targets and number of false answers for DV and RVIP and the LMT parameters, the difference between the 2 groups will be analysed using an analysis of variance on change from baseline with treatment, period, sequence and day as fixed effects, treatment*day as interaction and subject within sequence as random effect. In case of significant treatment*day interaction, an analysis of variance for a 2-period, cross-over design will be performed at each day.

For LMT parameters, the difference between the 2 groups will be analysed using an analysis of covariance on raw data with treatment, period, sequence and day as fixed effects, baseline as covariate, treatment*day as interaction and subject within sequence as random effect. In case of significant treatment*day interaction, an analysis of covariance for a 2-period, cross-over design will be performed at each day.

Pain model (cold pressor test)
CP_{tol}, pain VAS_{max}, pain VAS_{total} and the pain $AUC_{0\text{-}120s}$ will be described by treatment group.
The parameters will be compared at D6 between the 2 groups using an analysis of variance with fixed terms for sequence, period and treatment and a random term for subject within sequence. If necessary, data will be log-transformed.

Cough challenge (capsaicin)
Concentrations of capsaicin causing two (C2) and five coughs (C5) on the interval 0-15s will be described by treatment group and measurement time. Number of coughs on the interval 0-15s and 15-60s C2 and C5 on the interval 15s-60s will be also described.
C2 and C5 on the interval 0-15s will be compared at D7 between the 2 groups using an analysis of variance with fixed terms for sequence, period and treatment and a random term for subject within sequence. If necessary, data will be log-transformed.

IOP measurement
The intraocular pressure will be described by treatment group and measurement time.
IOP will be compared at D8 between the 2 groups using an analysis of variance with fixed terms for sequence, period and treatment and a random term for subject within sequence.

Apomorphine challenge

NS_{max}, NS t_{max} and NS $AUC_{(0\text{-}90\ mins)}$ will be described by treatment group.

NS_{max} and NS $AUC_{(0\text{-}90\ mins)}$ will be compared at D10 between the 2 groups using an analysis of variance with fixed terms for sequence, period and treatment and a random term for subject within sequence. If necessary, data will be log-transformed.

Moreover, NS t_{max} will be compared between the 2 treatment groups using a Wilcoxon signed ranks test.

14.2.8. Safety analysis

14.2.8.1. Criteria

- Adverse events
- Vital signs
- Standard 12-lead ECG
- Haematology, coagulation, biochemistry, urinalysis
- Physical examination
- Complete neurological examination (PD Part)

14.2.8.2. Statistical methodology

Analysis of safety parameters will be performed on the Safety set.

The description will be performed:

- by treatment group for SAD and MAD parts,
- by food condition for measurements during the treatment periods and overall for measurements performed at screening and end-of-study visits for FI part,
- by treatment group for measurements during the treatment periods and overall for measurements performed at screening and end-of-study visits for PD part,

Vital signs, ECG parameters, (raw data and changes from baseline) will be described.

Laboratory data (haematology, biochemistry, coagulation and urinalysis) will be described.

In addition, vital signs, ECG and laboratory data could be compared to potentially clinically significant range to be specified in the SAP.

All these parameters will be listed by subject and measurement time. Data that is out of normal ranges will be flagged.

All AEs, SAEs and AEs leading to withdrawal will be listed by subject.

A TEAE is an event that occurs after IMP dosing or that was present prior to dosing but became exacerbated after dosing (during a treatment period for FI and PD parts).

Repetitions will only be counted once in summary tables. Repetitions are defined as follows:

If a given subject presents several AEs with same verbatim text during the same treatment period, only one event is defined, the others are considered repetitions or recurring episodes. The start time of the event will be the start time of the first occurrence, the end time will be the end time of the last episode. The intensity will be the highest recorded intensity for all episodes. The causality will be the highest likelihood recorded for all episodes.

AEs will be summarized by system organ class and preferred term in tables with:

- The number of subjects with at least one adverse event and number of AEs for each treatment group/food condition and overall,
- The number of subjects with at least one TEAEs and number of TEAEs for each treatment group/food condition.

Analyses taking into account intensity and drug relationship to treatment could be also carried out.

15. RIGHT OF ACCESS TO DATA AND SOURCE DOCUMENTS

15.1. Monitoring

The investigator will allow the representative of the sponsor and the study Monitor:

- To inspect the site, the facilities and the material used for the study,
- To meet all members of the team involved in the study,
- To consult all the documents relevant to the study,
- To check that the CRFs have been correctly completed,
- To have direct access to source documents for comparison of data therein with the data in the CRFs,
- To check that AEs have been documented,
- To verify that the study is carried out in compliance with the protocol, GCP, and other relevant regulations.

This study will be monitored at regular intervals, by mutual agreement of the investigator and Monitor.

All information dealt with during these visits will be treated as strictly confidential.

The investigator will provide the sponsor with the following:

- Progress reports at regular intervals,
- Adequately completed CRFs.

15.2. Audit- Inspection

The Investigator will be informed that an audit may be carried out, at the request of the sponsor, before, during or after the study.

The Investigator will be informed that the Regulatory Authorities may also carry out an inspection. In this case, the Investigator must inform the Sponsor as soon as he receives the notification of inspection.

The Investigator must allow the representatives of the Regulatory Authorities and persons responsible for the audit to:

Inspect the site, facilities and material used for the study,

Meet all members of his team involved in the study,

Have direct access to study data and source documents,

Consult all the documents relevant to the study.

16. QUALITY CONTROL

The Investigator or the appointed persons agree to complete the subject's CRF sheets, at each investigation. Only the Investigator or appointed persons in his team may fill out or correct the CRFs. The CRFs will display the subject number corresponding to the order of inclusion in the study (4 digits) and the initials of the subject.

All corrections and alterations of data on the CRFs must be made by the Investigator or by the appointed persons in the following manner: strike through the datum to be corrected using a single line so that the original remains legible; correction fluid must never be used. The correction should be written to the side or above the original entry and must be initialled and dated by the person who makes the correction.

It is the responsibility of the monitor to make certain that the data are included in the CRFs is complete and accurate.

At the end of each study period, the investigator and the monitor must sign and date the CRF in order to attest respectively to:

- authenticity of the data collected in the CRF ,
- coherence between the data in the CRF and those in the source documents.

At the end of the study, the Investigator will keep a copy of the correctly completed CRFs for his own records and will organise the archiving of the original CRFs on behalf of the Sponsor.

The Investigator will keep a log of study volunteers screened for study participation and as appropriate, will indicate the reason individual study volunteers did not enter the study. The Investigator must submit to the Sponsor or its representatives a completed CRF for each subject who receives any IMP.

If computerised medical files are used, and if the computer system allows, no change made in the medical files by the Investigator should obscure the original information. The record must clearly indicate that a change was made and clearly provide a means to locate and read the prior information. The Investigator will save data at regular intervals.

The Investigator must guarantee the safety of the study data in the medical files by implementing security measures to prevent unauthorised access to the data and to the computer system.

17. STUDY SUSPENSION, TERMINATION, AND COMPLETION

The Sponsor may suspend or terminate the study or any part of the study at any time for any reason.

If the Investigator suspends or terminates the study, the Investigator will promptly inform the Sponsor and the regulatory authorities and provide them with a detailed written explanation. The Investigator will return all test articles, test article containers, and other study materials to the Sponsor.

Upon study completion, the Investigator will provide the sponsor and Independent Review Board (IRB)/ Independent Ethics Committee (IEC) with final reports and summaries as required by regulations. For Investigational New Drug application (IND) studies, the Investigator must submit a written report to the Sponsor and IRB/IEC within 3 months after the completion or termination of the study.

18. ETHICS AND REGULATORY ASPECTS

18.1. Current texts

The study will be carried out in accordance with:

- The text of the Declaration of Helsinki adopted by the World Medical Assembly in June 1964, amended in Tokyo 1975, in Venice 1983, in Hong-Kong 1989, in Somerset West 1996 updated with a clarification note in Edinburgh 2000 and Washington 2002, revised in Tokyo 2004, Seoul 2008 and Fortaleza 2013.
- The ICH recommendations: Good Clinical Practice (E6), (CPMP/ICH/135/95), 2002.
- Directive 2001/20/EC translated in French law of the 9th August 2004 (n°2004-806) and the decree of 26th April 2006 (n°2006-477) relative to biomedical research and French law n°2002-303 of 4th March 2002 regarding subject's rights.
- French law n°78-17 of 6th January 1978 relative to Data processing, Data files and individual liberties, modified by law n°2004-801 of 6th August 2004.
- European directive 2005/28/CE dated 8 April 2005 (GCP) translated in French law by the Decision of 24 Nov 2006 fixing the GCP for biomedical research on drugs for human use.
- "Guideline on the Investigation Of Bioequivalence" published by the Committee for Medicinal Products for Human Use (CHMP)
- Note for Guidance on the Investigation of Bioavailability and Bioequivalence (CPMP/EWP/QWP/1401/98)
- Guideline on strategies to identify & mitigate risks for First-in-human clinical trial with Investigational Medicinal Products, CHMP 01/09/07.

18.2. Subject Information and Consent

An unconditional prerequisite for a subject's participation in the trial is his/her written informed consent. The subject's written informed consent to participate in the trial must be given before any trial-related activities are carried out. Nevertheless, if necessary and after documented agreement of the Sponsor, any measures, identical to those planned in the protocol for subject's screening, already performed within the timelines given by the protocol for the screening exams, could be used for the protocol in order to minimize the constraints of subjects. This procedures can only be performed after a Biotrial generic written informed consent already approved by Ethics Committee has been signed by the subject and the investigator.

Subjects will be verbally informed by an investigator of all pertinent aspects of the trial: the nature of the study, its aim, its possible risks and restrictions, its duration and the fee that they will receive. The protocol will be explained during a meeting prior to the study and each subject must be informed that participation in the study is voluntary and that he/she may withdraw from the study at any time. At this meeting, an information sheet will be given to each subject. The language used in doing so must be chosen so that the information can be fully and readily understood by lay persons.

The subject should carefully read before signing and dating the informed consent form. He/she should be allowed to ask all necessary questions to the investigator. The informed consent form must be signed and personally dated by both the subject and the investigator. A copy of the signed document should be given to the subject. A copy will be kept for 15 years by the investigator (the second copy given to the subject).

The completed "subject / patient screen log" will be signed by the investigator to attest that consent has been obtained from all subjects.

Whenever important new information becomes available that may be relevant to the subject's consent, the written subject information sheet and any other written information provided to subjects will be revised by the sponsor and be submitted again to the IEC/IRB for review and favourable opinion/authorisation. The agreed, revised information will be provided to each subject in the trial for signing and dating. The Investigator will explain the changes to the previous version.

18.3. Submission to the authorities

18.3.1. Ethics Committee opinion

It is the responsibility of the Sponsor to seek and obtain the favourable opinion of the CPP (Independent Ethics Committee). This activity is delegated to Biotrial.

The present biomedical trial will not be initiated until this favourable opinion is obtained.

18.3.2. ANSM authorisation

It is the responsibility of the Sponsor to seek and obtain the ANSM (French Drug Agency) authorisation for conducting the present biomedical trial. This activity is delegated to Biotrial.

The present biomedical trial will not be initiated until the ANSM authorisation is received.

18.3.3. Protocol Amendments

Any significant change (substantial modification) in the study requires a protocol amendment for authorisation or for information. Concerning a protocol amendment sent to ANSM and/or CPP for authorisation, an investigator must not make any changes to the study without regulatory authorities and sponsor approval except when necessary to eliminate apparent immediate hazards to the subjects. A protocol change intended to eliminate an apparent immediate hazard to subjects may be implemented immediately, but the change must then be documented in an amendment, reported to the regulatory authorities/IEC within 5 working days, and submitted in the required time frame. All protocol amendments must be reviewed and approved following the same process as the original protocol.

18.4. Regulatory requirements

In compliance with French law approval as a site for biomedical research without direct individual therapeutic benefit was granted to by the Minister for Health to Biotrial Rennes centre (site n°05001M).

19. DATA PROCESSING AND ARCHIVING OF DOCUMENTS AND DATA RELATIVE TO THE RESARCH

After the study, the Investigator will keep all information relevant to the study for 15 years.

20. CONFIDENTIALITY AND AGREEMENTS

20.1. Confidentiality

Before starting the study, the Investigator must confirm receipt of adequate documentation from the Sponsor so as to be able to decide whether or not to perform the study.

All documents and information given to the Investigator by the Sponsor with respect to the study are strictly confidential.

The Investigator and his colleagues agree to use them only with the framework of this study, in order to carry out the protocol. This agreement is binding as long as the confidential information has not been disclosed to the public by the Sponsor.

The Investigator may use the technical protocol to obtain the informed consent of study subjects. It must not be disclosed to other parties without the written authorisation of the Sponsor.

The Investigator keeps a confidential subjects identification list for the study. The Investigator must maintain source documents for each subject in the study.

Data on subjects collected on CRFs during the study will be documented in an anonymous fashion. All information on CRFs must be traceable to these source documents.

20.2. Obligations of the Sponsor and contracting to CRO

Sponsor will:

- Assure that the Contract Research Organisation (CRO) to which they delegate their tasks is fully qualified and that the study location and the logistical means are adapted to and available for his requirements.
- Supply the Investigator with the following:
  - an up-to-date IB,
  - information on the IMP,
  - the exclusion period during which the person cannot participate in another study,
- Supply the IMP and the documentation, take charge of supplementary costs for specific apparatuses and supplies and of general running costs incurred for the study,

- Immediately forward to the Investigator any information that is liable to have direct consequences on the study.
- Immediately examine with the Investigator any serious adverse event and take the required steps to guarantee the security of the study participants.

The Sponsor will contract Biotrial to:

- Contract an insurance policy for the present project and provide the sponsor and the Investigator with a copy of the certificate,
- Ensure that the study file was submitted to the CPP and that the committee has given its approval,
- Transmit to the ANSM and CPP all the information concerning any new discovery concerning the conduct of the study or the IMP,
- Submit the Clinical Trial Application (CTA) to the ANSM and obtain the approval for the present biomedical trial.
- Inform ANSM and CPP of any serious adverse event as soon as he becomes aware of the fact.
- Inform ANSM and CPP of premature termination of the study and indicate the reason(s),
- Send to ANSM within the legal time limit, any CPP decision further to extra information submitted,
- Designate the qualified people who participate in the clinical research and guarantee their training,
- Designate the appropriate people or Committees who undertake the management and the supervision of the study, data collection, data management and statistical analysis and writing of the study report.

21. REPORT AND PUBLICATION

21.1. Report:

The results of the study will be reported in a CSR. This report will be prepared by Biotrial according to existing Standard Operating Procedures (ICH Biotrial or Sponsor Clinical Report).

In compliance with the regulations, the final report will be produced within one year of completing the study and in agreement with the work order.

The final report will be provided to the investigator.

The CSR will be provided to the sponsor as Word and PDF files. Also, SAS transfer files of the data will be provided electronically. All data will be presented CTD compliant.

21.2. Publication:

The Investigator will only use the information in the field of the study. The information cannot be used without sponsor's authorisation. Hence all or part of the information should only be divulged, submitted for publication or claim for industrial proprietary act with the written consent of Sponsor.

22. REFERENCES

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