Chiesi

CLINICAL STUDY PROTOCOL

STUDY CODE No.: CLI-06001AA1-02

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EVALUATION OF THE ABSOLUTE BIOAVAILABILITY AND MASS BALANCE OF CHF6001 FOLLOWING A SINGLE INHALED DOSE CO-ADMINISTERED WITH AN INTRAVENOUS RADIOLABELLED MICROTRACER DOSE IN HEALTHY VOLUNTEERS

Version No.: 1.0
Date: 19 October 2020

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma - Italy

GENERAL INFORMATION

SPONSOR:	Chiesi Farmaceutici S.p.A.* Via Palermo 26/A 43122 Parma - Italy + 39 0521 2791 *also reported as Chiesi thereafter
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INVESTIGATOR (or Coordinating Investigator)	[redacted], **MB ChB**
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CENTRAL BIOANALYTICAL LABORATORY:	[redacted]
CENTRAL LABORATORY FOR RADIOANALYSIS	[redacted]

VERSION HISTORY

Version	Date	Change History
1.0	*19 October 2020*	*First version*

PROTOCOL OUTLINE

Study title	Evaluation of the absolute bioavailability and mass balance of CHF6001 following a single inhaled dose co-administered with an intravenous radiolabelled microtracer dose in healthy volunteers
Sponsor	Chiesi Farmaceutici S.p.A. - Via Palermo 26/A 43122 Parma - Italy
Name of the Product	CHF6001
Centre(s)	Single centre
Indication	Chronic Obstructive Pulmonary Disease (COPD) (study conducted in healthy subjects)
Study design	Open-label, uncontrolled, non-randomized, single dose
Study phase	Phase I
Objectives	The objectives of this study will be: • To determine the absolute bioavailability of CHF6001 following a single inhaled dose co-administered with an intravenous microtracer dose • To characterize the mass balance and the elimination routes of CHF6001 after an intravenous microtracer dose • To characterize the relevant metabolites in plasma, urine and feces • To assess the safety and tolerability of the study treatment
Treatment duration	One single dose treatment
Test product dose/route/regimen	4 inhalations of CHF6001 800µg/20mg NEXThaler® DPI (total dose of 3200µg) co-administered with an intravenous microdose (18.5µg and 500nCi (18.5kBq)) of [^{14}C]-labelled CHF6001.
Number of subjects	8 subjects
Study population	Healthy male subjects
Inclusion/exclusion criteria	**Inclusion criteria**: *Subjects must meet all of the following inclusion criteria at screening, to be eligible for enrolment into the study:* 1. Subject's written informed consent obtained prior to any study-related procedure; 2. Able to understand the study procedures, the risks involved and ability to be trained to use correctly the inhalers and to generate sufficient PIF (at least 40 L/min) using the In-Check device set as per NEXThaler® inhaler resistance and Placebo inhaler; 3. Male subjects aged 30 to 55 years inclusive; 4. Body mass index (BMI) within the range of 18 to 35 kg/m^2 inclusive; 5. Non- or ex-smoker who smoked < 5 pack years (pack-years = the number of cigarette packs (20 cigarettes) or equivalent per day times the number of years) and who stopped smoking > 1 year prior to screening; 6. Good physical and mental status, determined on the basis of the medical history and a general clinical examination, at screening and before treatment; 7. Vital signs at screening within limits defined as: Diastolic Blood Pressure 40-90 mmHg and Systolic Blood Pressure 90-140 mmHg,

(three) measures performed in supine position after at least 5 minutes of resting. The average from triplicate measurements must be within the defined range;

Note: In case the values are outside the defined ranges, the assessment can be repeated once before treatment.

8. 12-lead digitised Electrocardiogram (12-lead ECG) in triplicate considered as normal (40 ≤ Heart rate ≤ 110 bpm, 120 ms ≤ PR ≤ 210 ms, QRS ≤ 120 ms, QTcF ≤ 450 ms) at screening visit. The average from triplicate measurements must be within the defined range;

   Note: In case of values outside the defined ranges, the assessment can be repeated once before treatment.

9. Lung function measurements within normal limits at screening (Normal values: $FEV_1/FVC > 0.70$ and FEV_1 >80% predicted);

   Note: In case the values are outside the defines values, the assessment can be repeated once before treatment.

10. Regular bowel movements (averaging one or more bowel movements per day) at screening;
11. Males fulfilling one of the following criteria:
    a. Males with non-pregnant WOCBP partners: they and/or their partner of childbearing potential must be willing to use a highly effective birth control method in addition to the male condom from the signature of the informed consent and until 90 days after the follow-up visit or
    b. Males with pregnant WOCBP partner: they must be willing to use male contraception (condom) from the signature of the informed consent and until 90 days after the follow-up visit or
    c. Non-fertile male subjects (contraception is not required in this case) or
    d. Males with partner not of childbearing potential (contraception is not required in this case).

Moreover, subjects must not donate sperm during the study and for 90 days after the follow-up visit. For the definition of WOCBP and of fertile men and the list of highly effective birth control methods, refer to Appendix 2 (or section 4.1 of the CTFG guidance for more detailed information).

Inclusion criteria 7, 8 and 9 could be checked again once before treatment.

- *If the criteria are met after the re-check, the subject can continue the study.*
- *If the criteria are still not met, the subject will be discontinued and screen failed.*

The inclusion criterion 6 will be re-checked at Day -1

- *If the criterion is met, the subject can continue the study.*
- *If the criterion is not met, the subject will be discontinued and screen failed.*

Exclusion criteria:

	The presence of any of the following exclusion criteria at screening will exclude a subject from study enrolment: 1. Participation in another clinical trial with an investigational drug in the 3 months or 5 half-lives of that investigational drug (whichever is longer) preceding the administration of the study drug; a longer and more appropriate time could be considered by the principal investigator based on the elimination half-life and/or long-term toxicity of the previous investigational drug; 2. Clinically relevant and uncontrolled respiratory, cardiac, hepatic (including Gilbert syndrome), gastrointestinal, renal, endocrine, metabolic, neurologic, or psychiatric disorder that may interfere with successful completion of this protocol according to Investigator judgement; 3. Clinically relevant abnormal laboratory values at screening suggesting an unknown disease and requiring further clinical investigation or which may impact the safety of the subject or the evaluation of the study results according to Investigator judgement; *Note: In case of abnormal laboratory values, that could indicate a temporary condition, the test can be repeated once before treatment.* 4. Subjects with history of breathing problems (e.g. history of asthma including childhood asthma); 5. Positive to HIV1 or HIV2 serology at screening; 6. Positive results from the Hepatitis serology which indicates acute or chronic Hepatitis B or Hepatitis C at screening (i.e. positive HB surface antigen (HBsAg), HB core antibody (IgM anti-HBc), HC antibody); 7. Blood donation or blood loss (equal or more than 450 mL) less than 2 months prior screening or prior to treatment; 8. Positive urine test for cotinine at screening or before treatment; 9. Documented history of alcohol abuse within 12 months prior to screening or a positive alcohol breath test at screening or before treatment; 10. Documented history of drug abuse within 12 months prior to screening or a positive urine drug screen at screening or before treatment; 11. Intake of non-permitted concomitant medications in the predefined period prior to screening or before treatment or the subject is expected to take non-permitted concomitant medications during the study (see “non-permitted concomitant medications” section); 12. Presence of any current infection, or previous infection that resolved less than 7 days prior to screening or before treatment; 13. Known intolerance and/or hypersensitivity to any of the excipients contained in the formulation used in the trial; 14. Unsuitable veins for repeated venipuncture; 15. Heavy caffeine drinker (> 5 caffeinated beverages e.g., coffee, tea, cola per day); 16. Abnormal haemoglobin level defines as <13mg/l for males and <11.3mg/l for female at screening; 17. Subjects using e-cigarettes within 6 months prior to screening;

	18. Subjects been involved in a study involving a ^{14}C-labeled drug within the 12 months prior to enrollment; 19. Subjects with exposure to significant diagnostic or therapeutic radiation (eg, serial X-ray, computed tomography scan, barium meal) or current employment in a job requiring radiation exposure monitoring within 12 months prior to Day-1; 20. Documented COVID-19 diagnosis within the last 8 weeks or which has not resolved within 14 days prior to screening and before treatment. *The exclusion criterion 3, if met at the screening visit, can be checked again once before the treatment:* • *If the criterion is not met after re-check, the subject can continue the study.* • *If the criterion is still met, the subject will be discontinued and screen failed.* *The following exclusion criteria 7, 8, 9, 10, 11, 12 and 20 will be re-checked at Day -1 visit:* • *If all criteria are not met, the subject can continue the study.* • *If at least one criterion is met, the subject will be discontinued and screen failed.*
Study plan	After the screening visit, which will take place between 3 to 31 days prior to dosing, the eligible subjects will enter the study that comprises one single dose treatment period. During the treatment period, the subjects will come to the clinical site on Day -1. On Day 1, they will be administered with 4 inhalations of CHF6001 NEXThaler® 800µg followed by a 15 min intravenous infusion of the tracer (injection of 10 mL [^{14}C]-CHF6001 1.85µg/mL) ending at the expected T_{max} of 2 h for the inhaled dose, giving a total infused dose of 18.5µg and 500nCi (18.5kBq). Inhaled drug administration will occur in fasting conditions (at least 10 hours fasting prior to administration) and the subjects will remain fasted until 2 hours post dose. The subjects will remain at the clinical site under permanent supervision of the medical staff, until the morning of Day 11. They will be discharged after the 240h post-dose (i.v.) assessments on Day 11 are completed. A follow-up phone call (or visit, if necessary) will be performed from 7 to 10 days after discharge or premature discontinuation to check and record concomitant medications and the status of any unresolved adverse events or any new adverse events
Most relevant allowed concomitant treatments	Occasional paracetamol (maximum 2 g per day with a maximum of 10 g per 14 days for mild conditions not meeting exclusion criteria).
Most relevant forbidden concomitant treatments	• Intake of any enzyme-inducing drugs, enzyme-inhibiting drugs, biologic drugs or any drug known to have a well-defined potential for hepatotoxicity (e.g. isoniazide, nimesulide, ketoconazole) are not permitted within 3 months prior to screening and throughout the study period (including the follow up period)

	• Intake of any drug treatment, including prescribed or OTC medicines, vitamins, homeopathic remedies etc., with the exception of the allowed concomitant medications specified in the relative section are not permitted within 14 days prior to screening and throughout the study period including the follow up period
Pharmacokinetics variables	<u>Following intravenous (IV) administration</u>: • Plasma [^{14}C]-CHF6001 $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$, V_z, V_{dss} and systemic clearance (CL) • Plasma [^{14}C]-total $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$ • Urine and feces [^{14}C]-CHF6001 and [^{14}C]-total excreted fraction (Fe_u, Fe_f and Fe_{u+f}) • Systemic blood clearance (CL_{blood}) [^{14}C]-CHF6001 • Renal plasma and blood clearance (CL_R and CL_{R_blood}) [^{14}C]-CHF6001 • Fraction of the relevant metabolites in plasma, and fraction of the relevant metabolites recovered in urine and feces (Fr_plasma, Fr_urine, Fr_feces, Fr_urine+feces) • Metabolized fraction (Fe_{met}) • Blood to plasma ratio ($R_{b/p}$) [^{14}C]-CHF6001 • Blood to plasma ratio ($R_{bt/pt}$) [^{14}C]-total • Hepatic extraction (E_{h_blood}) [^{14}C]-CHF6001 <u>Following inhaled administration</u>: • Plasma CHF6001 $AUC_{(0\text{-}t)\ inh}$, C_{max_inh}, t_{max_inh}, $AUC_{(0\text{-}\infty)\_inh}$, $t_{1/2\_inh}$, absolute inhaled bioavailability (F_{inh})
Measurement of PK variables	• **Blood samples** for plasma non-radiolabeled CHF6001 to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints post inhaled dose: 15, 30, 60, 90 min, 2, 3.75, 5.75, 7.75, 9.75, 11.75, 13.75, 25.75, 49.75, 73.75, 97.75, 121.75, 145.75, 169.75, 193.75, 217.75 and 241.75 hours. • **Blood samples** for plasma [^{14}C]-total, [^{14}C]-CHF6001, [^{14}C] of the relevant metabolites to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints relative to the start of the IV infusion: 5, 10, 15 [end of infusion], 20, 25, 30, 45, 60 min, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours. • **Blood samples** for whole blood [^{14}C]-total and [^{14}C]-CHF6001 at pre-dose (within 60 min from inhaled dosing) and at 20 min after the start of IV infusion. • **Urine samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from -12 hours to immediately before study drug inhaled administration

	(-12-0h) and at the following time frames relative to the start of the IV infusion: 0-4h, 4-8h, 8-12h, 12-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h. • **Fecal samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from check-in (Day-1) to immediately before study drug inhaled administration (check-in -0h) and at the following time frames relative to the start of the IV infusion: 0-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h. **Characterization of the relevant metabolites** For metabolites in plasma accounting for ≥10% of total radioactivity recovered in this matrix, structural characterization may be performed, where possible. For metabolites in urine and feces (total excreta) accounting for ≥10% of total administered radioactivity, structural characterization may be performed, where possible.
Safety variables	• Adverse Events (AEs) and Adverse Drug Reactions (ADRs). • Systolic (SBP) and Diastolic (DBP) Blood Pressure. • Heart rate (HR) from local 12-lead safety ECG. • Clinical laboratory evaluations (chemistry, haematology and fasting glucose)
Measurement of safety variables	• Vital Signs: blood pressure will be evaluated after 5 min in supine position at pre-dose and at 2,5 h post-dose • Local 12-lead Safety ECG: HR will be evaluated at pre-dose and at 2,5 hours post dose • Chemistry, haematology and fasting glucose at screening and Day 11
Sample size calculation	According to the exploratory nature of the study, no formal sample size calculation was performed. A total of 8 subjects will be included in the study to characterize the absolute bioavailability and the mass balance of CHF6001.
Statistical methods	**Pharmacokinetic variables** All PK variables (except for t_{max}, t_{max_inh} and t_{max_iv}) for plasma, urine and feces [^{14}C]-total, [^{14}C]-CHF6001 and non-radiolabeled CHF6001 will be summarized by means of descriptive statistics including n (number of observed values), arithmetic mean, standard deviation (SD), coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum. t_{max}, t_{max_inh}, t_{max_iv} will be summarized by using n, median, minimum and maximum. Concentration (mass equivalent per mL or mass per mL)/time curves for plasma [^{14}C]-total, [^{14}C]-CHF6001, and non-radiolabeled CHF6001 will be presented in linear/linear and log/linear scale. Plots will be presented based on arithmetic means and by subject (all individual plasma concentration/time curves in one graph).

	Bar charts based on arithmetic means showing the percentage of urine and feces excreted fraction for [^{14}C]-total, and [^{14}C]-CHF6001 in the different time intervals will be presented. Bar charts based on arithmetic means showing percentages of the relevant metabolites recovered in plasma, urine and feces will be presented. All individual concentration (mass equivalent per mL or mass per mL) data and PK parameters will be listed. In addition, a listing of the actual sampling times relative to drug inhalation will be provided. Concentrations (mass equivalent per mL or mass per mL) will be summarized by scheduled sampling time by using n, arithmetic mean, SD, CV, median, minimum and maximum. **Safety Variables** • The number and percentage of subjects experiencing adverse events, adverse drug reactions, serious adverse events and adverse events leading to study withdrawal will be summarized. • Percentage of subjects with clinical relevant changes at 2,5 hrs post-dose in SBP, DBP and HR (from 12-lead ECG) defined as o Change from pre-dose in DBP > 10 mmHg; o Change from pre-dose in SBP > 20 mmHg; o Change from pre-dose in in HR > 20 bpm will be summarized. • For quantitative laboratory parameters (chemistry, haematology and fasting glucose), shift tables from screening with regards to normal range will be presented.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADR	Adverse Drug Reaction
AE	Adverse Event
Ae	Excreted amount
ALT	Alanine Aminotransferase
AMS	Accelerator Mass Spectrometry
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical classification
AUC	Area Under the Curve
AUC_{0-t}	Area Under the Curve, from 0 to the last quantifiable concentration
$AUC_{0-\infty}$	Area Under the Curve, from 0 to infinite
BMI	Body Mass Index
BP	Blood Pressure
BTPS	Body Temperature and Pressure, Saturated
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
CL	Systemic Clearance
CL_{blood}	Total body blood clearance
CL_R	Renal plasma clearance
CL_{R_blood}	Renal blood clearance
C_{max}	Maximum of concentration
C_{min}	Minimum of concentration
COPD	Chronic Obstructive Pulmonary Disease
CRO	Contract Research Organization
CTFG	Clinical Trial Facilitation Group
CV	Coefficient of Variation
DBP	Diastolic Blood Pressure
DPI	Dry-powder inhaler
DRR	Data Review Report
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDTA	Ethylenediaminetetraacetic acid
E_{h_blood}	Hepatic extraction
EMA	European Medicines Agency
FDA	Food and Drug Administration
fe	Percent of the dose excreted over a sampling interval
Fe	Cumulative percent of dose excreted
FEV_1	Forced Expiratory Volume in the first second
F_{inh}	Absolute Inhaled Bioavailability
Fe_{met}	Metabolized fraction
Fr	Fraction of the relevant metabolites
FSH	Follicle-stimulating hormone
FVC	Forced Vital Capacity

GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease
γ-GT	Gamma-Glutamyl Transpeptidase
hAME	Human Absorption Metabolism and Excretion
Hb	Total haemoglobin
HB	Hepatitis B
Anti HBc	Hepatitis B core antibody
HBsAg	Hepatitis B surface Antigen
Hct	Haematocrit
HCV	Hepatitis C
HIV1/HIV2	Human Immunodeficiency Virus 1/Human Immunodeficiency Virus 2
HR	Heart Rate
HV	Healthy Volunteers
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	Inhaled Corticosteroid
IMP	Investigational Medicinal Product
IRB	Institutional Review Board
IV	Intravenous
K	Potassium
LABA	Long Acting β2-Agonist
LAMA	Long Acting Muscarinic Antagonist
LC+AMS	Liquid Chromatography-Accelerator Mass Spectrometry
LC-MS/MS	Liquid Chromatography Tandem Mass Spectrometry
LLOQ	Lower Limit Of Quantification
LRTI	Lower Respiratory Tract Infection
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum Tolerated Dose
Na	Sodium
OECD	Organisation for Economic Co-operation and Development
OTC	Over The Counter
PDE4	Phosphodiesterase-4
PIF	Peak Inspiratory Flow
PK	Pharmacokinetic
PLT	Platelets count
PR	Time Interval Between the P and R wave in the ECG
QRS	Time Interval Between the Q and R and S wave in the ECG
QTcF	Fridericia corrected Time Interval Between the Q and T wave in the ECG
RBC	Red Blood Cells
$R_{b/p}$	Whole blood to plasma ratio
SAAC	Short Acting Anticholinergics
SABA	Short Acting Beta2 Agonists
SAE	Serious Adverse Event

SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SD	Standard Deviation
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
t_{max}	Time to reach the C_{max}
t_{min}	Time to reach the C_{min}
$t_{½}$	Elimination half-life
TEAE	Treatment Emergent Adverse Event
TP	Treatment Period
Vdss	Volume of distribution at steady-state
Vz	Volume of distribution during the terminal phase
WBC	White Blood Cells
WHO	World Health Organization
WOCBP	Women of childbearing potential
WONCBP	Women of non-childbearing potential

Note:
"Chiesi Farmaceutici S.p.A." is also reported as "Chiesi" throughout the text.

CONTENT

1. INTRODUCTION

1.1 Background information

The pathogenesis and progression of chronic obstructive pulmonary disease (COPD) is, in part, due to chronic inflammation [1]. However, the nature and severity of inflammation in COPD varies, and pharmacological anti-inflammatory treatments are unlikely to be effective in all patients; a precision medicine approach is needed to selectively target patients to increase the chance of therapeutic success [2].

Phosphodiesterase-4 (PDE4) is an enzyme that mediates the breakdown of cyclic adenosine monophosphate (cAMP), with PDE4 inhibition having anti-inflammatory effects in a broad range of cell types. The orally administered PDE4 inhibitor roflumilast prevents exacerbations in patients with COPD [3], [4]. However, systemic exposure after roflumilast administration can cause side effects such as nausea, weight loss and gastrointestinal disturbance, which may limit its use in clinical practice [5].

CHF6001 is a novel inhaled PDE4 inhibitor [6], currently in clinical development that has been specifically designed as an extra-fine formulation to be delivered via inhalation and to have a low systemic exposure. This allows CHF6001 to reach therapeutic concentration in the target organ, the lung, yet reduces exposure in the systemic circulation thus limiting systemic adverse effects [7].

Safety, tolerability and the PK profile of CHF6001 were evaluated in healthy volunteers (HV) in two phase 1 trials in which doses up to 4800µg/day were administered up to 14 days. All doses of CHF6001 were safe and well tolerated, both after single and repeated administration. Neither apparent gastrointestinal intolerance nor cardiovascular safety signal was noted [8].

Clinical pharmacology, safety, tolerability and PK profile of CHF6001 were investigated in an allergen challenge model in mild asthmatic subjects [9] and in subjects with COPD [10] evaluating the anti-inflammatory effect of CHF6001 on sputum and blood biomarkers of inflammation. Doses up to 1200µg/day were administered in asthma subjects and up to 3200µg/day were administered to COPD subjects, for 28 days. CHF6001 did show a reduction (~30%) in late asthmatic response to allergen challenge in comparison with placebo. In COPD subjects, on maintenance treatment with triple therapy, CHF6001 significantly decreased a number of key biomarkers of airway inflammation in sputum and in blood [11].
This observed anti-inflammatory effect of CHF6001 translated in a consistent and relevant reduction of moderate or severe exacerbations of COPD in patients with chronic bronchitis, on maintenance therapy with a LABA, in a 6-month dose finding trial [12]. CHF6001 proved to be safe and well tolerated especially with regard gastro-intestinal side effects, a known PDE4 inhibitor ‘drug class’ effects.

The objective of the proposed study is to evaluate the bioavailability of CHF6001 after inhaled administration and to characterize the mass balance and route of elimination of CHF6001 in human along with its relevant metabolites.
For detailed information on preclinical and clinical data please refer to the Investigator's Brochure [13].

1.2 Study rationale

Human radiolabelled mass balance or absorption, distribution, metabolism and excretion (ADME) studies are required by regulatory agencies for the registration of new drug, and therefore, are an integral part of the drugs development programs.

[redacted]

This study along with data from the mass balance studies in toxicology species, provides essential information on the exposure of the parent compound and the metabolites of CHF6001.
It will be an open-label study in 8 healthy male participants aimed to assess the absolute bioavailability, the mass balance and routes of elimination of CHF6001 in humans, using [^{14}C]-radiolabelled drug substance administered as an intravenous (IV) infusion concomitantly to an inhaled non-radiolabelled dose.
The study involves a small amount of radioactivity; therefore, only adult males are included in the trial. It will be conducted as an open-label trial as the study measures are objective outcomes (eg, total radioactivity in select biological matrices). Conducting the study in healthy subjects will allow the evaluation of CHF6001 metabolism in the absence of concomitant medications. The dose, subject population, study duration, and sample collection timing are considered adequate to achieve the study objectives.

1.3 Risk/benefit assessment

A single inhaled dose of CHF6001 3200µg DPI will be administered to healthy subjects in an open-label fashion. This dose (and higher inhaled doses up to 4800µg) had been assessed after repeated dosing (14 days) in healthy subjects and had proved to be safe and well tolerated [8].
A microdose was selected because CHF6001 has not previously been administered by IV infusion to humans, although testing has been conducted in pre-clinical studies [13] and in order to evaluate the mass balance and absolute inhaled bioavailability in the same setting, thus reducing the overall exposure of healthy volunteers to CHF6001. The radioactive dose administered in this trial is an acceptable dose for [^{14}C]-labeled human drug metabolism studies of this type. It is expected that this dose will provide a sufficient radioactive signal for total radioactivity counting and quantitative radioprofiling of [^{14}C]-CHF6001 in plasma and excreta with minimal radiation risk to subjects.
The volunteers will be followed at the clinical site and closely monitored throughout the study period.

This trial is a mass balance study, so no benefits are expected for the healthy volunteers. Considering the safety profile of the IMP, the minimal radiation risk to subjects, the measures in place to assure the patients' safety and the expected scientific value, the overall risk/benefit assessment can be considered acceptable for the proposed trial.

This trial will be conducted in compliance with the Declaration of Helsinki (1964 and amendments) current ICH E6 Good Clinical Practices and all other applicable laws and regulations.

2. STUDY OBJECTIVES

- To determine the absolute bioavailability of CHF6001 following single inhaled dose co-administered with an IV microtracer dose
- To characterize the mass balance and routes of elimination of CHF6001 after an IV microtracer dose
- To characterize the relevant metabolites in plasma, urine and feces
- To assess the safety and tolerability of the study treatment

3. STUDY DESIGN

This clinical trial is a single centre Phase I study, with a single dose, non-randomized, open-label, uncontrolled design.

Screening

At screening visit (31 to 3 days before first study treatment administration), subjects will be selected to enter in the study according to the eligibility criteria. The signed informed consent form will be obtained prior to any study related procedures.

Treatment Period

During the treatment period, subjects will remain at the clinical site (all subjects will be treated at [redacted] site) from Day -1 (the day before the study drug administration) until the morning of Day 11. Each subject will fast from 10 hours pre-dose until 2 hours post-dose (between 2 hours post dose and discharge, standard meals will be provided at standard times as per site practice). They will be discharged after the 240h post-dose (i.v.) assessments are completed.

Follow-up

A follow-up phone call (or visit, if necessary) will be performed between 7 to 10 days after discharge or premature discontinuation to check and record concomitant medications and the status of any unresolved adverse events or occurrence of any new adverse events.

End of the trial

The end of the trial is defined at execution of the Follow-up call (or visit) of the last subject of the trial.

4. SUBJECT SELECTION CRITERIA

4.1 Subject Recruitment

A total of 8 subjects will be included in the study.

Subjects will be screened by selection from the subjects' database of the clinical centre or via advertisement. Study specific advertisement will be submitted to the Ethics Committee for approval, before using it in the study.

4.2 Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrolment into the study:

1. Subject's written informed consent obtained prior to any study-related procedure;
2. Able to understand the study procedures, the risks involved and ability to be trained to use correctly the inhalers and to generate sufficient PIF (at least 40 L/min) using the In-Check device set as per NEXThaler® inhaler resistance and Placebo inhaler;
3. Male subjects aged 30 to 55 years inclusive;
4. Body mass index (BMI) within the range of 18 to 35 kg/m^2 inclusive;
5. Non- or ex-smoker who smoked < 5 pack years (pack-years = the number of cigarette packs (20 cigarettes) or equivalent per day times the number of years) and who stopped smoking > 1 year prior to screening;
6. Good physical and mental status, determined on the basis of the medical history and a general clinical examination, at screening and before treatment;
7. Vital signs at screening within limits defined as: Diastolic Blood Pressure 40-90 mmHg and Systolic Blood Pressure 90-140 mmHg, (three) measures performed in supine position after at least 5 minutes of resting. The average from triplicate measurements must be within the defined range;
   Note: In case the values are outside the defined ranges, the assessment can be repeated once before treatment.
8. 12-lead digitised Electrocardiogram (12-lead ECG) in triplicate considered as normal (40 ≤ Heart rate ≤ 110 bpm, 120 ms ≤ PR ≤ 210 ms, QRS ≤ 120 ms, QTcF ≤ 450 ms) at screening visit. The average from triplicate measurements must be within the defined range;
   Note: In case the values are outside the defined ranges, the assessment can be repeated once before treatment.
9. Lung function measurements within normal limits at screening: FEV_1 >80% predicted and FEV_1/FVC > 0.70;
   Note: In case the values are outside the defined ranges, the assessment can be repeated once before treatment.
10. Regular bowel movements (averaging one or more bowel movements per day) at screening;
11. Males fulfilling one of the following criteria:
    a. Males with non-pregnant WOCBP partners: they and/or their partner of childbearing potential must be willing to use a highly effective birth control method in addition to the male condom from the signature of the informed consent and until 90 days after the follow-up visit or
    b. Males with pregnant WOCBP partner: they must be willing to use male contraception (condom) from the signature of the informed consent and until 90 days after the follow-up visit or
    c. Non-fertile male subjects (contraception is not required in this case) or
    d. Males with partner not of childbearing potential (contraception is not required in this case).

Moreover, subjects must not donate sperm during the study and for 90 days after the follow-up visit. For the definition of WOCBP and of fertile men and the list of highly effective birth control methods, refer to Appendix 2 (or section 4.1 of the CTFG guidance for more detailed information).

Inclusion criteria 7, 8 and 9 could be checked again once before treatment.

- *If the criteria are met after the re-check, the subject can continue the study.*

- *If the criteria are still not met, the subject will be discontinued and screen failed.*

The inclusion criterion n. 6 will be re-checked at Day -1.

- *If the criterion is met, subject can continue the study.*
- *If the criterion is not met, subject will be discontinued and screen failed.*

4.3 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrolment:

1. Participation in another clinical trial with an investigational drug in the 3 months or 5 half-lives of that investigational drug (whichever is longer) preceding the administration of the study drug; a longer and more appropriate time could be considered by the principal investigator based on the elimination half-life and/or long-term toxicity of the previous investigational drug;
2. Clinically relevant and uncontrolled respiratory, cardiac, hepatic (including Gilbert syndrome), gastrointestinal, renal, endocrine, metabolic, neurologic, or psychiatric disorder that may interfere with successful completion of this protocol according to Investigator judgement;
3. Clinically relevant abnormal laboratory values at screening suggesting an unknown disease and requiring further clinical investigation or which may impact the safety of the subject or the evaluation of the study results according to Investigator judgement;
   Note: In case of abnormal laboratory values, that could indicate a temporary condition, the test can be repeated once before treatment.
4. Subjects with history of breathing problems (e.g. history of asthma including childhood asthma);
5. Positive to HIV1 or HIV2 serology at screening;
6. Positive results from the Hepatitis serology which indicates acute or chronic Hepatitis B or Hepatitis C at screening (i.e. positive HB surface antigen (HBsAg), HB core antibody (IgM anti-HBc), HC antibody;
7. Blood donation or blood loss (equal or more than 450 mL) less than 2 months prior screening or prior to treatment;
8. Positive urine test for cotinine at screening or before treatment;
9. Documented history of alcohol abuse within 12 months prior to screening or a positive alcohol breath test at screening or before treatment;
10. Documented history of drug abuse within 12 months prior to screening or a positive urine drug screen at screening or before treatment;
11. Intake of non-permitted concomitant medications in the predefined period prior to screening or before treatment) or the subject is expected to take non-permitted concomitant medications during the study (see “non-permitted concomitant medications” section);
12. Presence of any current infection, or previous infection that resolved less than 7 days prior to screening or before treatment;
13. Known intolerance and/or hypersensitivity to any of the excipients contained in the formulation used in the trial;
14. Unsuitable veins for repeated venipuncture;
15. Heavy caffeine drinker (> 5 caffeinated beverages e.g., coffee, tea, cola per day);
16. Abnormal haemoglobin level defines as <13mg/l for males and <11.3mg/l for female at screening;
17. Subjects using e-cigarettes within 6 months prior to screening;
18. Subjects been involved in a study involving a ^{14}C-labeled drug within the 12 months prior to enrollment;

19. Subjects with exposure to significant diagnostic or therapeutic radiation (eg, serial X-ray, computed tomography scan, barium meal) or current employment in a job requiring radiation exposure monitoring within 12 months prior to Day-1;
20. Documented COVID-19 diagnosis within the last 8 weeks or which has not resolved within 14 days prior to screening and before treatment.

The exclusion criterion 3, if met at the screening visit, can be checked again once before the treatment:

- *If the criterion is not met after the re-check, the subject can continue the study.*
- *If the criterion is still met, the subject will be discontinued and screen failed.*

The following exclusion criteria 7, 8, 9, 10, 11, 12 and 20 will be re-checked at Day -1 visit:

- *If all the criteria are not met, the subject can continue the study.*
- *If at least one criterion is met, the subject will be discontinued and screen failed.*

4.4 Subject Withdrawals

Subjects must be discontinued from the study for any of the following reasons:

- An adverse event occurs that, in the opinion of the investigator, makes it unsafe for the subject to continue in the study. In this case, the appropriate measures will be taken.
- The subject is lost to follow-up.
- The subject withdraws consent.
- The subject's safety is affected by violation of inclusion or exclusion criteria or use of not-permitted concomitant medication.
- The subject is unwilling or unable to adhere to the study requirements, i.e., non-compliance.
- The sponsor or the regulatory authorities or the Ethics Committee(s), for any reason, terminates the entire study, or terminates the study for this trial site or this particular subject.

It is understood by all concerned that an excessive rate of withdrawals can render the study uninterpretable; therefore, unnecessary withdrawals of subjects should be avoided.
However, should a subject discontinue the study, all efforts will be made to complete and report the observations as thoroughly as possible.

In case of withdrawal, the Investigator must fill in the “Study Termination” page in the eCRF, reporting the main reason for withdrawal.

In case actual drop-out rate is higher than expected, additional subjects may be enrolled in order to reach a suitable number (at least 6) evaluable subjects.

If a subject is withdrawn/drops-out of the study after receiving the test treatment, the subject study number and corresponding test treatments should not be reassigned to another subject.

5. CONCOMITANT MEDICATIONS

5.1 Permitted concomitant Medications

Occasional paracetamol (maximum 2 g per day with a maximum of 10 g per 14 days for mild conditions not meeting exclusion criteria).

5.2 Non-permitted concomitant Medications

- Intake of any enzyme-inducing drugs, enzyme-inhibiting drugs, biologic drugs or any drug known to have a well-defined potential for hepatotoxicity (e.g. isoniazide, nimesulide, ketoconazole) are not permitted within 3 months prior to screening and throughout the study period (including the follow up period)

- Intake of any drug treatment, including prescribed or OTC medicines, vitamins, homeopathic remedies etc., with the exception of the allowed concomitant medications specified in the relative section are not permitted within 14 days prior to screening and throughout the study period including the follow up period

In case of intake of any forbidden concomitant medication during the study the need for this subject to be withdrawn from the study will be carefully evaluated by the Investigator on the basis of the potential impact on the study outcome and/or safety evaluation in the subject's best interest.

6. TREATMENT(S)

The non-radiolabelled IMP will be supplied to the clinical site under the responsibility of the Sponsor, who will also provide the Pharmacist/Investigator with appropriate certificates of analytical conformity and QP release.
The radiolabelled Drug substance (DS) will be provided by [redacted], [redacted] will manufacture the Drug Product (DP) for IV infusion, it will be manufactured and Certified by a [redacted] QP and supply straight to the clinic.
The analytical and microbiological methods related to the radiolabelling procedure will be validated and reports will be generated.

6.1 [redacted]

CHF6001 800µg/20mg NEXThaler® DPI

- *Active ingredient*: CHF6001
- [redacted]

CHF6001 DPI NEXThaler® matched placebo (for Training kit)

- *Active ingredient:* None
- [redacted]

[^{14}C]-CHF6001 1,85µg/ml (50nCi/ml) solution for IV infusion

- *Active ingredient*: [^{14}C]-CHF6001
- *Formulation vehicle*: ethanol and kolliphor®HS 15 in 0.9% w/v saline
- *Appearance*: clear and colourless solution in a glass vial

6.2 Dosage and Administration

6.2.1 Selection of doses in the study

Inhaled dose

The highest CHF6001 dose planned to be tested in the Phase 3 program studies is 1600µg administered twice-daily corresponding to a total daily dose of 3200µg.

[redacted]

CHF6001 is mainly metabolized to CHF5656 and CHF6095, which are detected in the circulation of COPD patients at concentrations approximately 20- and 50- fold lower, respectively, than the parent compound CHF6001. CHF5956, the major metabolite of CHF6001, was 2,000-fold less potent than the parent compound in an in vitro model of LPS-induced TNF-alpha production in human isolated peripheral blood mononuclear cells.

Therefore, this clinical trial was designed taking into consideration the linear and time-independent PK at concentrations found to be safe and well tolerated in healthy subjects and patients for the active parent compound CHF6001. For this reason, mass balance characterization of CHF6001 after single dose administration of 3200µg is deemed appropriate.

Intravenous dose

The proposed intravenous dose of 18.5µg (given over a 15-minutes infusion) accounts for less than 0.6% of the concomitant cold inhaled dose (3200µg). The overall dose (3218.5µg) is therefore expected to be well within the highest dose tested in humans (4800µg by inhalation).
The radioactive dose will be approximately 500nCi. The radioactive burden is considered to pose negligible additional risk above the background cosmic radiation, so no dosimetry estimation is necessary.

6.2.2 Dosage

One single dose administration of CHF6001 will be given to all subjects, as follows:

CHF6001 DPI Inhalation:

- 4 inhalations of CHF6001 800µg/20mg DPI NEXThaler® giving a total dose of 3200 µg CHF6001

[^{14}C]-CHF6001 IV infusion:

- Detailed information on the process for formulation preparation are in the pharmacy protocol. The IV formulation will be administered intravenously as a single dose over 15 minutes, ending at the expected T_{max} of 2 h for the inhaled dose giving a total infused dose of 18.5µg and 500nCi (18.5kBq).

6.2.3 Administration

The treatment administration will take place at the clinical site in the morning of Day 1 of the treatment period, according to the instruction for use (provided in separate documents) and under the supervision of the investigator or his/her designee.
In the morning of Day 1, before treatment administration, restrictions related to the study drug administration will be applied, as reported in section 7.1.4.

6.2.3.1 DPI administration

The study medication will be administered using the DPI device.

For inhalation treatments, time 0 is defined as the moment when the first inhalation takes place. Subjects will take the medication in an upright position. Additionally, the subjects will be instructed to hold their breath for 10 secs following each inhalation and to wait 50 seconds before taking the next inhalation, in order to have 1 min between consecutive inhalations. A delay of ± 5 seconds will not be considered as a protocol deviation.

The dose counter of the NEXThaler® device should be inspected before and after each inhalation.

Detailed Instruction for use of the NEXThaler device for IMP use will be provided in a separate document.

Treatment evidence will be recorded in the eCRF and in the administration log, by the investigator or designee. Any issues occurred during the inhalations, evaluated in this sense by the investigator or designee, will be reported in the eCRF. An incorrect inhalation is defined as “a significant reduction of delivered dose that reaches the lungs based on investigator judgement”. Even if this is the case, no extra inhalations (i.e., more than the 4 inhalations required) will be administered.

In order to prevent any kind of contamination, the administration of the drug will take place in a ventilated room well separated from the blood sampling room. Subjects will remain in the room of administration only for the time strictly needed for the administration. Furthermore, during inhalation, subjects and assistant will wear special protective coats and gloves, which will be removed before entering the blood sampling room. The assistant administering the inhalations will not be allowed to participate in the blood sampling procedures on Day 1.

6.2.3.2 IV administration

[^{14}C]-CHF6001 will be administered according to the pharmacy protocol to participants at the site by a 15 min intravenous infusion ending at the expected T_{max} of 2 h for the inhaled dose administered. Administration will be documented in the source documents and reported in the eCRF.

6.2.4 Subject Training

6.2.4.1 Training with In-Check DIAL

At screening and on Day -1 and Day 1 pre-dose of the treatment Period, the In-Check DIAL will be used to train subjects to use the DPI inhaler correctly. In-Check DIAL is a PIF meter that simulates the internal resistance of the device, to enable the measurement of airflow as if the subject is using the device. The In-Check DIAL resistance selector will be aligned with the internal resistance of the NEXThaler® device. In order to ensure an optimum aerosol dispersion threshold, a PIF of at least 40 L/min is required. The optimal PIF is 60 L/min. Subjects who will reach the required PIF at the NEXThaler® setting, will be considered able to generate sufficient PIF for a correct inhalation with the NEXThaler®.

Following three successful inhalations with the In-Check DIAL device, the subjects will be asked to continue using the same inspiratory flow during drug inhalation. Detailed instructions for use of In-Check DIAL are reported inside the commercial package.

Subject will not be treated if the training is not completed with success. Training evidence will be recorded in the eCRF including the three "PIF" values.

6.2.4.2 Training with DPI placebo

At screening and on Day-1 and Day1 pre-dose of the treatment period, the correct use of the DPI device (NEXThaler®) will be explained to the subject according to the instruction for use (provided in separate documents).

Subject will be trained using placebo inhalers identical to the devices used for the administration of study drug. Therefore, the training will be done with DPI device, repeating inhalation for approximately 4 times.

At screening, and on Day -1 of the treatment period, the subject will receive a new training kit. The training kit dispensed at Day -1 will be used to follow the training procedure at Day -1 and Day 1 before the administration of the study treatment.
Subject will not be treated if the training is not completed with success. Training evidence will be recorded in the administration log and in the eCRF, by the Investigator or designee.

6.3 Packaging

All investigational products will be prepared in accordance with Good Manufacturing Practices (GMP) as required by the current Good Clinical Practices (GCP). *Chiesi Farmaceutici S.p.A.* will supply the non-radiolabelled DPI material while [redacted] will supply the radiolabelled IV solution as described in the following sections.

6.3.1 Training Kit

The training kits will be supplied to the Investigator as one DPI training box for each subject, containing one placebo DPI inhaler.

- Primary packaging: 1 labelled inhaler of CHF6001 Placebo NEXThaler® dry powder for inhalation
- Secondary packaging: 1 labelled pouch containing 1 labelled inhaler of CHF6001 Placebo NEXThaler®
- Tertiary packaging: Box containing 1 pouch containing 1 labelled inhaler of CHF6001 Placebo NEXThaler®

6.3.2 Treatment Kit DPI

Treatment kit will be supplied to the Investigator as one treatment kit for each subject.

- Primary packaging: 1 labelled inhaler of CHF6001 NEXThaler® dry powder for inhalation
- Secondary packaging: 1 labelled pouch containing 1 labelled inhaler of CHF6001 NEXThaler®
- Tertiary packaging: Box containing 1 pouch containing 1 labelled inhaler of CHF6001 NEXThaler®

6.3.3 Treatment Kit [14C]-CHF6001 IV infusion

The radiolabelled Drug substance (DS) will be provided by [redacted], [redacted] will manufacture the Drug Product (DP) for IV infusion supply straight to the clinic and the treatment kit will be composed by one glass vial containing the [^{14}C]-CHF6001 IV formulation.
Further information can be found in the pharmacy protocol.

6.4 Labeling

All labeling will be in local language and according to local law and regulatory requirements and will be compliant with Annex 13 to the Volume 4 of the GMP.

6.5 Treatment allocation

All subjects will be administered one single dose (4 inhalations) of CHF6001 800µg/20mg DPI NEXThaler® according to a non-randomized, open-label design co-administered with an intravenous microtracer dose.

6.6 Treatment Code

No treatment code will be available since the study is performed according to a non-randomized design.

6.7 Treatment compliance

For each subject, the number of correct and incorrect inhalations taken during the treatment period will be recorded in the source documents and reported in the eCRF.

6.8 Drug Storage

The Pharmacist/Investigator or designee will be responsible for the safe storage of all medications assigned to this study, in a secure place with restricted access, and maintained within the appropriate ranges of temperature.

DPI training kits: initial storage of pouched inhalers "Requires no special conditions". Once removed from the pouch, the inhalers for training should be kept at ambient temperature but not above 30°C. At this temperature condition the residual shelf life will be six months (180 days). Therefore, the Pharmacist/Investigator at the site must write the use-by-date on the training kit labels once the inhalers are removed from the pouch. The use-by-date corresponds to the dispensing date plus 6 months**.** Please note that the use-by-date must not exceed the total shelf life of the product.

DPI treatment kits: initial storage of pouched inhalers: "Requires no special conditions". Once removed from the pouch, the inhalers for treatment should be kept at ambient temperature but not above 25°C. At this temperature condition the residual shelf life will be six weeks (42 days).

All treatment and training kits, must be used by the subject only at the clinical site under Investigator/Pharmacist supervision.

[^{14}C]-CHF6001 IV infusion treatment kits: The solution for IV infusion will be stored between 2 to 8°C. Further information can be found in the pharmacy protocol.

6.9 Drug Accountability

The Investigator, or the designated/authorized representative, is responsible for the management of all the study medications to be used for the study. Study medications that should be stored in a locked, secure storage facility with access limited to those individuals authorized to dispense the study medications.
An inventory will be maintained by the Investigator or pharmacist (or other designated individual), to include a signed account of all the study medication(s) received and dispensed during the trial.
At the conclusion or termination of the study, the Investigator or the pharmacist shall conduct and document a final drug supply (used and unused) inventory. An explanation will be given for any discrepancies.
All the study medications supplied, used or unused, will be destroyed directly from the Investigator by the centre. In this case, a destruction certificate must be asked to the investigational centre and filed both at site and at Sponsor. Destruction will not occur until authorized by Chiesi.

6.10 Provision of additional care

N.A.

7. STUDY PLAN

7.1 Study Schedule

	Screening	Treatment Period				Follow-up
		Day -1	Day 1	Day 2-10	Day 11	
Informed consent	X					
Residential period		X	X	X		
Discharge					X	
Treatment intake						
IMP administration			X[1]			
Training						
Training with In-Check Dial	X	X	X			
Training with Placebo	X	X	X			
Subject Health Evaluation						
In/Ex Criteria	X	X				
Medical History	X					
Demographic data	X					
Height and weight	X					
Alcohol breath test	X	X				
Physical examination	X	X			X[2]	
Adverse Events recording	X	X	X	X	X	X
Restrictions	X	X	X	X	X	
Concomitant Med	X	X	X	X	X	X
Safety assessment in blood						
Clinical Chemistry	X				X[2]	
Serology	X					
Haematology	X				X[2]	
Fasting glucose	X				X[2]	
Generic assessments in urine						
Urinalysis	X					
Drug panel	X	X				
Cotinine	X	X				
Samples for PK evaluations [a]						
Blood			X	X	X	
Urine		X	X	X	X	

	Screening	Treatment Period				Follow-up
		Day -1	Day 1	Day 2-10	Day 11	
Feces		X	X	X	X	
Cardiac assessments [b]						
Local ECG	X		X			
Vital Signs - Blood Pressure	X		X			
Pulmonary Assessment						
Spirometry	X					

Footnotes for Evaluation Schedule:

1. There will be 2 types of administration:
   - 4 inhalations of CHF6001 800µg/20mg DPI NEXThaler®
   - Intravenous infusion for 15min of the tracer starting 1h45min after the inhaled dose (time of the first inhalation) and ending at the expected T_{max} of 2 h for the inhaled dose
2. A full physical examination and collection of blood safety test will be performed at discharge. A follow-up visit will be done only if deemed necessary by the principal investigator.

a) Pharmacokinetic samples will be collected as:

- **Blood samples** for plasma non-radiolabeled CHF6001 to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints post inhaled dose**: 15, 30, 60, 90 min, 2, 3.75, 5.75, 7.75, 9.75, 11.75, 13.75, 25.75, 49.75, 73.75, 97.75, 121.75, 145.75, 169.75, 193.75, 217.75 and 241.75 hours.**
- **Blood samples** for plasma [^{14}C]-total, [^{14}C]-CHF6001, [^{14}C] of the relevant metabolites to be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints relative to the start of the IV infusion: **5, 10, 15 [end of infusion], 20, 25, 30, 45, 60 min, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours.**
- **Blood samples** for whole blood [^{14}C]-total and [^{14}C]-CHF6001 at pre-dose (within 60 min from inhaled dosing) and **20 min after start of infusion**
- **Urine samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from -12 hours to immediately before study drug inhaled administration (-12-0h) and at the following time frames relative to the start of the IV infusion: **0-4h, 4-8h, 8-12h, 12-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h**.
- **Fecal samples** for assessments of [^{14}C]-total, [^{14}C]-CHF6001 and [^{14}C] of the relevant metabolites to be collected at pre-dose from check-in (Day -1) to immediately before study drug inhaled administration (check-in -0h) and at the following time frames relative to the start of the IV infusion: **0-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h**.

b) Cardiac assessments will be performed at Screening and on Day 1 the treatment period as:

- **Local 12-lead ECG:** A triplicate ECG will be done for assessing eligibility at screening and a single ECG will be performed for general safety at predose and at 2,5 hours post dose (inhaled administration).
- **Vital Signs - BP**: BP measurement will be performed in triplicate at the following time points: at Screening and, for general safety, at predose and at 2,5 hours post dose (inhaled administration).

7.1.1 Screening

A Screening visit will be carried out from 3 to 31 days prior to the first administration of the study drug, in order to identify eligible consenting subjects for the study.

The following procedures will take place:

- Collection of the signed written INFORMED CONSENT by potential eligible subjects, after the study has been fully explained by the investigator or designee. The investigator or his/her designee should provide the participants ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial;
- SUBJECT SELECTION: inclusion/exclusion criteria will be checked
- SUBJECT TRAINING: subjects will be trained on a proper use of the inhaler with the In-Check Dial and the DPI Nexthaler® placebo.
- MEDICAL/SURGICAL HISTORY AND CONCOMITANT DISEASES: subject health history will be recorded.
- DEMOGRAPHY DATA COLLECTION: demographic data including age, gender and race, will be recorded.
- HEIGHT AND WEIGHT: will be recorded.
- ALCOHOL BREATH TEST: will be performed
- PHYSICAL EXAMINATION: a comprehensive physical examination will be performed.
- ADVERSE EVENTS RECORDING: AEs occurred since the signature of the informed consent will be recorded. In case of any clinically significant abnormality revealed during the physical examination or screening procedures, it will be recorded in the subject's medical history, unless its start date/time is after the informed consent signature date/time and it is not due to a pre-existing condition. In the latter case, it will be recorded as an adverse event.
- STUDY RESTRICTIONS: study restrictions criteria will be checked.
- PRIOR and CONCOMITANT MEDICATIONS CHECK: Any enzyme-inducing drugs, enzyme-inhibiting drugs, biologic drugs or any drug known to have a well-defined potential for hepatotoxicity (e.g. isoniazide, nimesulide, ketoconazole) taken in the last 3 months and any drug treatment, including prescribed or OTC medicines, vitamins, homeopathic remedies taken in the last 2 weeks will be recorded.
- BLOOD TESTING: blood samples collection for safety evaluations (clinical chemistry, haematology, serology, fasting glucose), according to section 7.2.2. Subjects have to be in fasting condition from at least 10 hours before blood samples are collected.
- URINE TESTING: urine samples collection for urinalysis, drug panel and cotinine test will occur according to section 7.2.3.
- LOCAL SAFETY ECG: Triplicate 12-lead ECG will be used to measure heart rate (HR) and will occur according to section 7.2.5.
- BLOOD PRESSURE: measurement of Systolic and Diastolic Blood Pressure (SBP and DBP) will occur according to section 7.2.4.
- SPIROMETRY: Lung function (FEV_1 (L), FEV_1 % of predicted normal value, FVC (L), FVC % of predicted normal value, FEV_1/FVC) will be measured according to section 7.2.6.

7.1.2 Treatment Period

The subject will arrive at the clinical site on Day -1 and will stay overnight until the accomplishment of the 240 hours post-dose (i.v.) study procedures. All subjects will be discharged on Day 11.

- On Day -1, subjects will be hospitalised and will undergo the planned assessments and checks to confirm eligibility)
- On Day 1 of treatment period, subjects will undergo pre-dose assessments, then will receive the treatment and will start post-dose assessments.
- From Day 2 until Day 11, subjects will continue the post-dose assessments. Subjects will be instructed to continue to follow study restrictions (see section 7.1.4). They will then be discharged soon after the 240h post-dose (i.v.) assessment.

The following procedures and assessments will take place during this period:

- SUBJECT TRAINING with devices: subjects will be trained on a proper use of the inhaler with In-Check Dial (Day -1/Day 1) and with the DPI placebo training kit (Day -1 and Day 1 pre-dose)
- SUBJECT SELECTION (Day -1): Inclusion criterion 6 and Exclusion criteria 7, 8, 9, 10, 11, 12 and 20, will be re-checked as requested in section 4.2 and 4.3. Subjects not fulfilling the mentioned criteria will be Screen Failed
- ALCOHOL BREATH TEST (Day -1): will be performed
- PHYSICAL EXAMINATION (Day 1): a full physical examination will be performed and any new revealed clinically significant abnormality since the screening visit will be recorded as an AE. Furthermore, a full physical examination will be performed at discharge.
- ADVERSE EVENTS RECORDING (Day -1 to Day 11): Any new AE/SAE occurred since the screening visit will be checked and recorded in the eCRF. The status of ongoing AEs will be checked and updated in the eCRF when applicable. Moreover, during this period in case of any new clinically significant abnormality revealed during the examination or procedures, it will be recorded as an adverse event.
- STUDY RESTRICTIONS (Day -1 to Day 11): study restrictions criteria will be checked.
- CONCOMITANT MEDICATIONS CHECK (Day -1 to Day 11): concomitant medications taken by the subject in the last 14 days and during the treatment period will be checked and recorded in the eCRF.
- BLOOD TESTING (at discharge): blood samples collection for safety evaluations (clinical chemistry, haematology, fasting glucose), according to section 7.2.2. Subjects have to be in fasting condition from at least 10 hours before blood samples are collected.
- URINE TESTING (Day -1): urine samples collection for drug panel and cotinine test will occur according to section 7.2.3.
- IMP ADMINISTRATION (Day 1)
- PK INVESTIGATION: Collection of blood (Day 1 to Day 11), urine and fecal samples (Day -1 to Day 11) for PK assessment will occur according to section 7.2.1.
- LOCAL SAFETY ECG: (Day 1) A single 12-lead ECG measurement will be performed on Day 1, at predose and at 2,5 hours post dose.
- BLOOD PRESSURE (Day 1): measurement of Systolic and Diastolic Blood Pressure (SBP, DBP) will occur according to section 7.2.4.

7.1.3 Follow-up

A follow-up call will be performed from 7 to 10 days after the discharge to perform the following:

- CONCOMITANT MEDICATIONS CHECK: concomitant medications taken to treat AEs by the subjects since the discharge will be checked and recorded in eCRF.
- ADVERSE EVENTS RECORDING: the status of unresolved AEs/SAEs at the discharge will be checked and any new AEs/SAEs occurred after the discharge will be recorded in the eCRF.

This safety follow-up call shall also be performed in case of premature discontinuation (7-10 days from the last administration intake).
If needed, according to the investigator's opinion, a follow-up visit can be performed.

7.1.4 Study restrictions and standardization

1. The following restriction must be applied during the study and must be checked at clinical unit from starting of Screening to discharge:
    - No smoking or intake of substances containing nicotine: within the entire study period.

2. The following restriction must be applied prior to the screening:
    - No strenuous activities: within 24h prior to the start of screening until the end of the visit.

3. The following restrictions must be applied during the treatment period (pre- and post-dose refer to the inhaled dose):

    - No food intake: within 10h pre-dose until 2h post-dose of study treatment drug. If needed, participants may consume prunes or prune juice to facilitate stool samples
    - No fluid intake: within 1h pre-dose and 1h post-dose of study treatment drug. Afterwards, in order to maintain adequate hydration, subjects should take at least 240 mL of water every 2h for the following 6h
    - No strenuous activities: within 24h pre-dose until Day 11
    - No intake of beverages or food containing alcohol: within 48h pre-dose until discharge on Day 11
    - No intake of beverages or food containing xanthine (coffee, tea, chocolate, cola), or grapefruit (neither fruit nor juice): within 48h pre-dose until discharge on Day 11. Decaffeinated products are permitted except during fasting periods
    - No intake of food containing poppy seeds: within 24h pre-dose until Day 11

4. The following restriction must be applied to prior to the screening and discharge:
    - No food intake: within 10h prior to the blood collection for the lab test.

If these restrictions are not respected, the visit can be rescheduled once and all the information regarding restriction(s) not respected, will be recorded in the eCRF. If the restrictions are again not respected, the visit will be performed anyway and all the information regarding restriction not respected will be recorded in the eCRF.

7.2 Investigations

In case of coinciding investigations, the time point for blood collection for PK evaluation takes priority over any other scheduled study activities, e.g. blood pressure and local safety ECG. Where other activities are scheduled together with the PK blood collection, these will be performed in a sequence allowing for blood sampling exactly at the scheduled time point. The exact time of each activity will be recorded in the eCRF.

7.2.1 Sample collection for PK evaluation and samples preparation

The laboratory analysis will be carried out following the GCP and the applicable principles of Good Laboratory Practice (GLP) regulations of the Organisation for Economic Co-operation and Development (OECD).

The analytical procedures will be described in a separate analytical protocol. Validation data and details of the analytical procedures will be gathered in an analytical report that will be included in the clinical study report.

The breakdown of blood samples collection is reported in Appendix 3.

7.2.1.1 Determination of CHF6001 and related analytes in human plasma

Blood samples for the determination of plasma total [^{14}C], [^{14}C]-CHF6001, metabolite profiling (if required) and non-radiolabeled CHF6001, will be collected and processed according to an established laboratory manual at pre-dose and in the 0-240h interval after dosing, as follows:

- 22 blood samples of 4 mL for plasma non-radiolabeled CHF6001 will be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints post inhaled dose: 15, 30, 60, 90 min, 2, 3.75, 5.75, 7.75, 9.75, 11.75, 13.75, 25.75, 49.75, 73.75, 97.75, 121.75, 145.75, 169.75, 193.75, 217.75 and 241.75 hours;
- 25 blood samples of 5 mL for plasma total [^{14}C] and [^{14}C]-CHF6001 will be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints relative to the start of the IV infusion: 5, 10, 15 [end of infusion], 20, 25, 30, 45, 60 min, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours;
- 25 blood samples of 10 mL for plasma [^{14}C] of the relevant metabolites will be collected at pre-dose (within 60 min from inhaled dosing) and at the following timepoints relative to the start of the IV infusion: 5, 10, 15 [end of infusion], 20, 25, 30, 45, 60 min, 2, 4, 6, 8, 10, 12, 24, 48, 72, 96, 120, 144, 168, 192, 216 and 240 hours;
- 2 blood samples of 2 mL for whole blood total [^{14}C] and [^{14}C]-CHF6001 will be collected at pre-dose (within 60 min from inhaled dosing) and 20 min after the start of infusion (for a total of 4 samples).

The processing, storage and shipment of samples are detailed in the laboratory manual document.

Day	Clock time (exemplary)	Time after inhaled administration	Time after start of i.v. administration	Time deviation allowed	PK timepoint inhaled administration	PK timepoint i.v. administration
1	08:00	Pre-dose		Within 60 minutes from inhalation	X[1]	X[1]

	09:00	**Start of inhaled administration**				
	09:15	15 min		± 2 min	X[1]	
	09:30	30 min		± 2 min	X[1]	
	10:00	60 min		± 5 min	X[1]	
	10:30	90 min		± 5 min	X[1]	
	10:45		**Start of infusion (SOI)**			
	10:50		SOI + 5 min	± 2 min		X[1]
	10:55		SOI + 10 min	± 2 min		X[1]
	11:00	2 h	SOI + 15 min **(end of infusion)**	± 2 min	X[1]	X[1]
	11:05		SOI + 20 min	± 2 min		X
	11:10		SOI + 25 min	± 2 min		X
	11:15		SOI + 30 min	± 2 min		X
	11:30		SOI + 45 min	± 5 min		X
	11:45		SOI + 60 min	± 5 min		X
	12:45	3.75 h	SOI + 2 h	± 5 min	X	X
	14:45	5.75 h	SOI + 4 h	± 10 min	X	X
	16:45	7.75 h	SOI + 6 h	± 10 min	X	X
	18:45	9.75 h	SOI + 8 h	± 10 min	X	X
	20:45	11.75 h	SOI + 10 h	± 20 min	X	X
	22:45	13.75 h	SOI + 12 h	± 20 min	X	X
2	10:45	25.75 h	SOI + 24 h	± 20 min	X	X
3	10:45	49.75 h	SOI + 48 h	± 1 h	X	X
4	10:45	73.75 h	SOI + 72 h	± 1 h	X	X
5	10:45	97.75 h	SOI + 96 h	± 1 h	X	X
6	10:45	121.75 h	SOI + 120 h	± 1 h	X	X
7	10:45	145.75 h	SOI + 144 h	± 1 h	X	X
8	10:45	169.75 h	SOI + 168 h	± 1 h	X	X
9	10:45	193.75 h	SOI + 192 h	± 1 h	X	X
10	10:45	217.75 h	SOI + 216 h	± 1 h	X	X
11	10:45	241.75 h	SOI + 240 h	± 1 h	X	X

SOI: Start Of Infusion
[1]: Fasted condition

Longer time deviations will be evaluated on a case by case basis during the Data Review Meeting.

Metabolite profiling: pooling across timepoints for each subject will be done. This will be followed

by chromatography (LC+AMS) and radio-peaks will be detected and quantified. For metabolites in plasma accounting for ≥10% of total radioactivity recovered in this matrix structural characterization may be performed, where possible.

7.2.1.2 Determination of CHF6001 and related analytes in human urine

Urine samples for total [^{14}C], metabolite profiling (if required) and [^{14}C]-CHF6001 assessment will be collected at pre-dose from -12 hours to immediately before study drug inhaled administration (-12-0h) and at the following time frames relative to the start of the IV from pre-dose infusion: 0-4h, 4-8h, 8-12h, 12-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h, for a total of 14 urine collection intervals.

Subjects will be asked to empty the bladder before both dosing and before the end of each collection interval. All urine voided during the collection intervals will be retained. Access to the toilet will be controlled by the site staff.

Collection and processing are described in the laboratory manual document.

The following time deviations from theoretical post-dose times related to the end of the collection interval will be allowed:

For 4 and 8 hours post-dose	± 10 min
For 12 and 24 hours post-dose	± 20 min
For >24 hours post-dose	± 30 min

Longer time deviations will be evaluated on a case by case basis during the Data Review meeting.

Metabolite profiling: pooling across timepoints and subjects will be done. This will be followed by chromatography (LC+AMS) and radio-peaks will be detected and quantified. For metabolites in urine and feces (total excreta) accounting for ≥10% of total administered radioactivity structural characterization may be performed, where possible.

7.2.1.3 Determination of CHF6001 and related analytes in human feces

Fecal samples for total [^{14}C], metabolite profiling (if required) and [^{14}C]-CHF6001 assessment will be collected at pre-dose from check-in (Day -1) to immediately before study drug inhaled administration (check-in -0h) and at the following time frames relative to the start of the IV infusion: 0-24h, 24-48h, 48-72h, 72-96h, 96-120h, 120-144h, 144-168h, 168-192h, 192-216h and 216-240h, for a total of 11 fecal collection intervals.

The feaces samples will be pooled across the defined time windows. The time of each void will be recorded and then assigned to the appropriate collection interval. Collection and processing are described in the laboratory manual document.

Metabolite profiling: pooling across timepoints and subjects will be done. This will be followed by chromatography (LC+AMS) and radio-peaks will be detected and quantified. For metabolites in urine and feces (total excreta) accounting for ≥10% of total administered radioactivity structural characterization may be performed, where possible.

7.2.2 Blood collection for safety evaluation

The following blood samples will be collected at Screening and at discharge:

- **3.5 mL** of blood sample for serum **clinical chemistry testing** (creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), Gamma-glutamyl transpeptidase (γ-GT), total bilirubin, alkaline phosphatase, sodium (Na), potassium (K)) and **fasting glucose**.
- **3.5 mL** of blood sample for serum **serology testing** (HIV1, HIV2, Hepatitis B and Hepatitis C) will be collected only at Screening.
- **4 mL** of blood sample for **haematology testing** (red blood cells count (RBC), white blood cells count (WBC) and differential (neutrophils, eosinophils, lymphocytes, monocytes) (% and values), total haemoglobin (Hb), haematocrit (Hct), platelets count (PLT)).

Below indications will be followed for safety evaluation in blood at screening:

- As for Exclusion criteria no. 3, in case clinically relevant abnormal laboratory values are observed, suggesting a temporary condition and requiring further clinical investigation, subject will be asked to return to clinic once before treatment, to perform again the testing measurement. If at that time no abnormalities are observed, subject can be included in the study, otherwise subject will be discharged and recorded as Screening Failure.

Blood collection and sample preparation will be performed according to procedures provided by the local laboratory which will be in charge to transmit the results and the laboratory normal ranges for entry in the eCRF.

7.2.3 Urine collection for safety evaluation

The following urine sample will be collected at Screening:

- urine sample for **urinalysis – quantitative** (proteins) and **urinalysis – qualitative** (ketones, microscopic examination of the sediment)
- urine sample for **drug test** (cannabinoids, opiates, cocaine, benzodiazepines, amphetamines, barbiturates)
- urine sample for **cotinine test**

The following urine samples will be collected on Day-1 of the Treatment Period:

- urine sample for **drug test** (cannabinoids, opiates, cocaine, benzodiazepines, amphetamines, barbiturates)
- urine sample for **cotinine test**

Urine collection and sample preparation will be performed according to procedures provided by the local laboratory which will be in charge to transmit the results to the Investigator for entry in the eCRF.

7.2.4 Blood Pressure

At Screening and on Day 1 of the treatment period (before blood sampling) Systolic and Diastolic Blood Pressure (SBP, DBP) will be measured in triplicate, within 5 minutes, at the following time points**: pre-dose and at 2,5 hours** after inhaled administration, for safety reasons. Subjects should

be resting in a quiet supervised setting with minimal stimulation (e.g. no television, loud music, computer games) and lay in a supine position for 5 minutes (whenever possible) before each measurement. No time deviations will be recorded for BP. Below indications will be followed for Blood Pressure measurement at Screening:

- As for Inclusion criterion no. 7, in case the values are out of the defined ranges (average of the triplicate parameters out of normal range i.e. Diastolic Blood Pressure 40-90 mmHg, Systolic Blood Pressure 90-140 mmHg), subject will repeat the Blood Pressure measurement. If the repeated values (the average of the triplicate parameters) are within the allowed ranges, subject can be included in the study, otherwise subject will be discharged and recorded as Screening Failure.

The single values and the average of triplicate Blood Pressure measurements will be reported in eCRF, and the average will be used to assess the inclusion criterion at screening.

7.2.5 Local Safety 12-Lead ECG

A local triplicate 12-lead digitalized Electrocardiogram (12-lead ECG) recording will be done and considered for safety purpose at Screening (before Blood pressure and blood sampling) to check the the subject eligibility (according to exclusion criterion) and a local single 12-lead ECG will be performed on Day 1 of the study period at **predose and at 2,5 hours** after inhaled administration. No time deviations will be recorded for ECG.

Subjects should be resting in a quiet supervised setting with minimal stimulation (e.g. no television, loud music, computer games) and lay in a supine position for 5 minutes (whenever possible) before each measurement.

Below indications will be followed for Local ECG measurement at Screening:

- As for Inclusion Criterion no. 8, in case abnormalities are observed with Local ECG ($40 \leq$ Heart rate ≤ 110 bpm, 120 ms $\leq$ PR ≤ 210 ms, QRS ≤ 120 ms, QTcF ≤ 450 ms for males) subject will perform again the ECG measurement. If at that time the average of the triplicate ECG parameters is within the allowed ranges, subject can be included in the study, otherwise subject will be discharged and recorded as Screening Failure.

Below indications will be followed for local ECG measurement during the treatment period:

- A single 12-lead ECG measurement will be performed on Day 1, at predose and at 2 hours post dose (in resting conditions as much as possible)

The values of all ECG parameters and the overall ECG evaluation will be reported in eCRF, at Screening and the average will be used to assess the inclusion criterion.

7.2.6 Lung function evaluation

The lung function measurements will be assessed locally at Screening.

Spirometry will be done according to the recommendation of the Official Statement of the European Respiratory Society and American Thoracic Society [17], [18]. Lung function measurements will be done with subjects either standing or sitting with the nose clipped after at least 10 minutes rest. Values will be corrected for BTPS conditions.

The following parameters will be recorded:

- Forced Expiratory Volume in the 1st second (FEV_1, L)
- Forced Vital Capacity (FVC, L)
- FEV_1 % of predicted normal value
- FVC % of predicted normal value
- FEV_1/FVC ratio

Predicted values of FEV_1 will be calculated according to formulas reported by Quanjer et al [19].

For FEV_1 and FVC, **the highest value from three technically satisfactory attempts** will be recorded in the eCRF (irrespective of the curve they come from). The chosen value should not exceed the next one by more than 150 mL. If the difference is larger, up to 8 measurements will be made and the largest value be reported. In order to be considered as technically satisfactory attempts, measurements should be free from cough and false-start. FEV_1/FVC should be taken from the highest FEV_1 and FVC values (even if not coming from the same curve).

Below indications will be followed for lung function measurement at screening:

- As for Inclusion Criterion no. 9, in case the criterion is not met, subject will repeat the test. If at that time the FEV_1/FVC >0.70 and FEV_1 >80% predicted, subject can be included in the study, otherwise subject will be discharged and recorded as Screening Failure.

The FEV1/FVC values will be provided as ratio. For eligibility purposes, values will be considered as acceptable if FEV1/FVC >0.70.

8. PHARMACOKINETIC ASSESSMENTS

Main objective of this study is to assess the absolute bioavailability, the mass balance and routes of elimination of CHF6001, using [^{14}C]-radiolabelled drug substance administered as an intravenous (IV) infusion concomitantly to an inhaled non-radiolabelled dose, in healthy volunteers.

Therefore, the following variables will be derived:

Following intravenous (IV) administration:

- Plasma [^{14}C]-CHF6001, $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$, V_z, V_{dss} and systemic clearance (CL)
- Plasma [^{14}C]-total $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$
- Urine and feces [^{14}C]-CHF6001and [^{14}C]-total excreted fraction (Fe_u, Fe_f and Fe_{u+f})
- Systemic blood clearance (CL_{blood}) [^{14}C]-CHF6001
- Renal plasma and blood clearance (CL_R and CL_{R_blood}) [^{14}C]-CHF6001
- Fraction of the relevant metabolites in plasma, and fraction of the relevant metabolites recovered in urine and feces (Fr_plasma, Fr_urine, Fr_feces, Fr_urine+feces)
- Metabolized fraction (Fe_{met})
- Blood to plasma ratio ($R_{b/p}$) [^{14}C]-CHF6001
- Blood to plasma ratio ($R_{bt/pt}$) [^{14}C]-total
- Hepatic extraction (E_{h_blood}) [^{14}C]-CHF6001

Following inhaled administration:

- Plasma CHF6001 $AUC_{(0\text{-}t)\_inh}$, C_{max_inh}, t_{max_inh}, $AUC_{(0\text{-}\infty)\_inh}$, $t_{1/2\_inh}$, absolute inhaled bioavailability (F_{inh})

The pharmacokinetic analysis will be performed using WinNonlin Phoenix 6.2 or later (Pharsight Corporation, Palo Alto, CA, USA). Actual elapsed time from dosing will be used to estimate all individual PK parameters. Standard non-compartmental methods will be used for the calculation of the following parameters from the individual plasma drug concentrations versus time profile.

The following PK parameters will be calculated, whenever possible, for each subject based on the whole blood concentrations of [^{14}C]-CHF6001, plasma concentrations of CHF6001, plasma [^{14}C]-CHF6001 and plasma [^{14}C]-total radioactivity:

- $AUC_{0\text{-}t}$: the area under the concentration-time curve from 0 to the last quantifiable concentration will be computed using the linear trapezoidal rule with linear interpolation;
- $AUC_{0\text{-}\infty}$: the area under the concentration-time curve extrapolated to infinity will be calculated as the sum of $AUC_{0\text{-}t}$ and a residual part extrapolated to infinite time. The residual area from the last concentration data point to infinite time is calculated using the approximation:

  $$\int_t^{\infty} C_t \mathrm{d}t = C_t / \lambda z$$

  Where λz is the first order terminal rate constant, estimated by linear regression of the terminal log-linear segment of the plasma log-concentration curve, and C_t is the last quantifiable concentration data point. In order to determine λz by log-linear regression, at least 3 points will be used. The quality of log-linear regression and the reliability of extrapolated PK parameters should be demonstrated by an adjusted coefficient of determination R^2 of at least 0.85;
- C_{max} and t_{max}: the value and time of the maximum concentration will be obtained directly from the experimental data without interpolation;
- $t_{½}$: the terminal half-life, associated with negative slope (-λz), calculated as 0.693/λz from the individual drug concentrations versus time profiles.
- $Dose_{IV}$: is the actual IV dose calculated as [weight of full syringe (g) minus syringe weight after dose (g)] multiplied by nominal concentration (µg/g)
- F_{inh}: inhaled absolute bioavailability based on $AUC_{(0\text{-}\infty)}$ for CHF6001 calculated as $F_{inh} = (AUC_{0\text{-}\infty\_inh} \times Dose_{iv}) / (AUC_{0\text{-}\infty\_iv} \times Dose_{inh})$. If $AUC_{0\text{-}\infty}$ is not calculable $AUC_{0\text{-}t}$ will be used.
- $R_{b/p}$: whole blood to plasma ratio is determined on the [^{14}C]-CHF6001 whole blood and plasma concentration (C_b and C_p) 20 min after the start of infusion, calculated as $R_{b/p}=C_b/C_p$
- $R_{bt/pt}$: whole blood to plasma ratio is determined on the total-[^{14}C] whole blood and plasma concentration (C_{bt} and C_{pt}) 20 min after the start of infusion, calculated as $R_{bt/pt}=C_{bt}/C_{pt}$
- CL: the total body plasma clearance following IV administration, calculated only for [^{14}C]-CHF6001 as $Dose_{IV}/AUC_{0\text{-}\infty}$
- CL_{blood}: the total body blood clearance following IV administration, calculated only for [^{14}C]-CHF6001 as $CL_{blood} = CL/R_{b/p}$
- V_{dss}: Volume of distribution at steady-state will be calculated only for [^{14}C]-CHF6001 as $Dose_{IV} \times AUMC_{0\text{-}\infty} / (AUC_{0\text{-}\infty})^2$

- V_z: the volume of distribution during the terminal phase, calculated only for [^{14}C]-CHF6001 as $Dose_{IV}/(AUC_{0-\infty} \times \lambda z)$
- Fr_plasma: fraction of the relevant metabolites in plasma calculated as 100 x AUC [^{14}C]-metabolite/ AUC [^{14}C]-total radioactivity

The following PK parameters will be calculated, whenever possible, for each subject based on the concentrations of urine [^{14}C]-CHF6001 and urine [^{14}C]-total radioactivity:

- Ae_u: amount excreted in urine (expressed in weight) over sampling interval calculated as: $Ae = C_{ur} \times V_{ur}$, where C_{ur} is the concentration in urine and V_{ur} is the volume of urine
- Cumulative Ae_u: cumulative amount excreted in urine, calculated as the sum of the amount excreted in urine for each collection period
- fe_u: percent of the dose excreted in urine over a sampling interval, where $fe_u = 100 \times (Ae_u/Dose_{IV})$
- Fe_u: cumulative percent of dose excreted in urine, calculated as the sum of the percent of dose excreted in urine for each collection period
- CL_R: renal plasma clearance calculated only for [^{14}C]-CHF6001, if plasma $AUC_{0-\infty}$ is calculable then CL_R = Cumulative $Ae_u/AUC_{0-\infty}$, if Plasma $AUC_{0-\infty}$ is not calculable then CL_R= Cumulative Ae_u/AUC_{0-t}.
- CL_{R_blood}: renal blood clearance calculated only for [^{14}C]-CHF6001, where $CL_{R\_blood} = CL_R/R_{b/p}$
- Fr_urine: fraction of the relevant metabolites recovered in urine calculated as amount recovered $(Ar_u)/Dose_{IV} \times 100$

The following PK parameters will be calculated, whenever possible, for each subject based on plasma [^{14}C]-CHF6001 and urine [^{14}C]-CHF6001 radioactivity:

- E_{h_blood}: hepatic extraction calculated from intravenous systemic and renal blood clearance ($CL_{blood} = CL/R_{b/p}$) and $CL_{R\_blood} = CL_R/R_{b/p}$, respectively and human hepatic blood flow (Q_H; assumed to be 87 l/h [20]); $E_{h\_blood} = (CL_{blood} - CL_{R\_blood})/Q_H$

The following PK parameters will be calculated, whenever possible, for each subject based on the concentrations of feces [^{14}C]-CHF6001 and feces [^{14}C]-total radioactivity:

- Ae_f: amount excreted in the feces over sampling interval as Ae_f = (concentration in faecal collected sample x collected sample weight) for each faecal collection interval
- Cumulative Ae_f: cumulative amount excreted in feces, calculated as the sum of the amount excreted in feces for each collection period
- fe_f: percent of the dose excreted in feces over a sampling interval, where $fe_f = 100 (Ae_f/dose_{IV})$
- Fe_f: cumulative percent of dose excreted in feces, calculated as the sum of the percent of dose excreted in feces for each collection period
- Fr_feces: fraction of the relevant metabolites recovered in feces calculated as amount recovered $(Ar_f)/Dose_{IV} \times 100$

The following PK parameters will be calculated, whenever possible, for each subject based on the concentrations of urine and feces [^{14}C]-CHF6001 and urine and feces [^{14}C]-total radioactivity:

- Cumulative Ae_{u+f}: cumulative amount excreted in urine and feces, calculated as Cumulative Ae_u + Cumulative Ae_f
- Fe_{tot}: cumulative percent of dose excreted in urine and feces, calculated as $Fe_u + Fe_f$
- Fr_urine+feces: fraction of the relevant metabolites recovered in urine and feces calculated as Fr_urine + Fr_feces
- Fe_{met}: Metabolized fraction calculated as Fe_{met} = [cumulative Ae_{u+f} (^{14}C-total) – cumulative Ae_{u+f} ([^{14}C]-CHF6001)]/ cumulative Ae_{u+f} (^{14}C-total)

9. SAFETY ASSESSMENTS

The safety profile will be assessed through the monitoring of the following:

- Adverse Events (**AEs**) and Adverse Drug Reactions (**ADRs**)
- **Vital signs** (Systolic and Diastolic Blood Pressure). Vital signs will be evaluated pre-dose and at 2,5 hours post-dose
- Heart Rate (HR) from **local 12-lead safety ECG** will be performed at pre-dose and at 2,5 hours post-dose
- **Clinical laboratory evaluations:** Blood chemistry, haematology and fasting glucose collected at Screening and Discharge

10. ADVERSE EVENT REPORTING

10.1 Definitions

An **Adverse Event** is "any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment".
An adverse event can therefore be any unfavorable and unintended sign (including abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An **Adverse Drug Reaction** is an "untoward and unintended responses to an investigational medicinal product related to any dose administered".
All adverse events judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression "reasonable causal relationship" means to convey in general that there are facts (evidence) or arguments meant to suggest a causal relationship.
The definition covers also medication errors and uses outside what is foreseen in the protocol, including misuse and abuse of the product.

A **Serious Adverse Event (SAE)/Serious Adverse Drug Reaction** is any untoward medical occurrence or effect that at any dose falls in one or more of the following categories:

- Results in death

Death is not an adverse event but an outcome. It is the cause of death that should be regarded as the adverse event. The only exception to this rule is "sudden death" where no cause has been established; in this latter instance, "sudden death" should be regarded as the adverse event and "fatal" as its reason for being serious.

- **Is life-threatening**

Life-threatening refers to an event in which the subject was at risk of death at the time of the event (e.g., aplastic anaemia, acute renal failure, and anaphylaxis). The term does not refer to an event which hypothetically might have caused death if it were more severe.

- **Requires hospitalisation or prolongation of existing hospitalisation**

Hospitalization refers to a situation whereby an AE is associated with unplanned formal overnight admission into hospital, usually for purpose of investigating and/or treating the AE. Hospitalization for the treatment of a medical condition that occurs on an "elective" or "scheduled" basis or for a pre-existing condition that did not worsen during the study should not necessarily be regarded as a AE. Complications that occur during the hospitalisation are AEs. If a complication prolongs hospitalisation, the event is an SAE. Emergency room visits that do not result in a formal admission into hospital should be evaluated for one of the other seriousness criteria (e.g., life-threatening; persistent or significant disability or incapacity; medically significant).

- **Results in persistent or significant disability or incapacity.**

The term significant disability should be viewed as any situation whereby an AE has a clinically important effect on the subject's physical or psychological well-being to the extent that the subject is unable to function normally.

- **Is a congenital anomaly or birth defect**

- **Is a medically significant adverse event**

This criterion allows for any situations in which important adverse events/reactions that are not immediately life-threatening or do not result in death or hospitalisation may jeopardise the subject's health or may require intervention to prevent one of the above outcomes.
Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalisation; or development of drug dependency or drug abuse.
Medical and scientific judgment should be exercised in deciding whether an event is serious because medically significant.

Any suspected transmission via a medicinal product of an infectious agent is also considered a serious adverse reaction.

A **Non-Serious Adverse Event/Non-Serious Adverse Drug Reaction** is an adverse event or adverse drug reaction that does not meet the criteria listed above for a serious adverse event/serious adverse drug reaction.

10.2 Expectedness

An expected adverse reaction is an adverse reaction, the nature or severity of which is consistent with the applicable reference safety information (included in the Investigator's Brochure for CHF6001), otherwise it is considered unexpected.

Reports which add significant information on specificity or severity of a known, already documented serious adverse drug reaction constitute unexpected events. For example, an event more specific or more severe than described in the reference safety information would be considered as "unexpected".

Examples of such events are: (a) acute renal failure as a labelled ADR with a subsequent new report of interstitial nephritis and (b) hepatitis with a first report of fulminant hepatitis.

10.3 Intensity of Adverse Event

Each Adverse Event must be rated on a 3-point scale of increasing intensity:

Mild: The event causes a minor discomfort or does not interfere with daily activity of the subject or does not lead to either modification of test treatment dosage or establishment of a correcting treatment.

Moderate: The event perturbs the usual activity of the subject and is of a sufficient severity to make the subject uncomfortable. The event leads to a diminution of dosage of the test treatment, or a temporary interruption of its administration or to the establishment of a correcting treatment.

Severe: The event prevents any usual routine activity of the subject and causes severe discomfort. It may be of such severity to cause the definitive interruption of test treatment.

10.4 Causality Assessment

The following "binary" decision choice will be used by the Investigator to describe the causality assessment:

- Reasonable possibility of a relatedness
- No reasonable possibility of relatedness

The expression "reasonable possibility of relatedness" is meant to convey, in general, that there are facts (evidence) or arguments meant to suggest a causal relationship.

The Investigator will be asked to consider the following before reaching a decision on causality assessment:

- Time relationship between study drug intake and event's onset;
- Dechallenge (did the event abate after stopping drug?);
- Rechallenge (did the event reappear after reintroduction?);
- Medical history;
- Study treatment(s);
- Mechanism of action of the study drug;
- Class effects;
- Other treatments-concomitant or previous;
- Withdrawal of study treatment(s);
- Lack of efficacy/worsening of existing condition;
- Erroneous treatment with study medication (or concomitant);
- Protocol related process.

10.5 Action taken with the study drug due to an AE

- Dose not changed
- Drug permanently withdrawn
- Drug temporarily interrupted
- Unknown
- Not applicable

10.6 Other actions taken

- Specific therapy/Medication
- Concomitant Procedure

10.7 Outcome

Each Adverse Event must be rated by choosing among:

- Recovered/resolved
- Recovering/resolving
- Not recovered/not resolved
- Recovered with sequelae/resolved with sequelae
- Fatal
- Unknown

10.8 Recording Adverse Events

All Adverse Events occurring during the course of the study must be documented in the Adverse Event page of the electronic Case Report Form (eCRF). Moreover, if the Adverse Event is serious, the Serious Adverse Event Form must also be completed.
It is responsibility of the Investigator to collect all adverse events (both serious and non-serious) derived by spontaneous, unsolicited reports of subjects, by observation and by routine open questionings.
The recording period for Adverse Events is the period starting from the Informed Consent signature until the subject's study participation ends.
Clinically significant abnormalities detected at Screening not due to a pre-existing condition or clinically significant changes at the following visits in the medical opinion of the investigator must be reported as adverse events in the eCRF.

If a clinically significant abnormal laboratory finding or other abnormal assessment meets the definition of an AE, then the AE eCRF page must be completed, as appropriate. A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the clinically significant abnormal laboratory finding, should be reported on AE eCRF page. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded.

For pharmacovigilance purposes, all SAEs should be followed-up in order to elucidate as completely and practically as possible their nature and/or causality until resolution of all queries, clinical recovery is complete, laboratory results have returned to normal, stable condition is reached or until the subject is lost to follow-up. Follow-up may therefore continue after the subject has left the study. In this case, the follow-up will continue with no timelines for related SAEs, while for unrelated SAEs the type and extent of follow-up undertaken will be determined for each individual case and will depend upon the nature (e.g. events with poor prognosis or which do not resolve), severity and medical significance of the event.

10.9 Reporting Serious Adverse Events to Chiesi

The Investigator must report all Serious Adverse Events to the [redacted] Safety Contact listed below within 24 hours of awareness. The information must be sent by providing the completed paper Serious Adverse Event form. At a later date, the [redacted] Safety Contact will report all information to Chiesi Global Pharmacovigilance, the Clinical Project Manager and the Clinical Research Physician.

Name and Title	Telephone no.	Fax no.	E-mail
[redacted] Safety Contact	Not applicable	[redacted]	[redacted]
Chiesi Safety Contact [redacted]	[redacted]	[redacted]	[redacted]

- Reporting of SAEs from the investigator site is from the time of subject's signature of informed consent and until the subject's study participation ends. After this date, even if no active monitoring of subjects is required, SAEs occurring to a subject should be reported if the investigator becomes aware of them.
- Up to the closure of the site, SAE reports should be reported to the [redacted] Safety Contact. New serious adverse events occurring after the site is closed should be reported directly to the Chiesi Safety Contact.

10.10 Reporting Serious Adverse Events to Regulatory Authorities/Ethics Committees/IRB

All SUSARs, which occur with the investigational medicinal products within or outside the concerned clinical trial, if required, will be reported in compliance with the timelines and standards for reporting SUSARs set out in the EU Directive 2001/20/EC [Directive 2001/20/EC of the European parliament and of the council of 4/April/2001] and linked guidance [European Commission, Enterprise and Industry Directorate General: Detailed guidance on the collection, verification and presentation of adverse event/reaction reports arising from clinical trials on medicinal products for human use, latest version]. The EMA and the concerned national health authority (if applicable) will be informed through Eudravigilance, or according to local requirements (as applicable) while the Ethics Committees and the investigators by CIOMS I form or by periodic line- listings produced by Chiesi Global Pharmacovigilance.
With regard to regulations in force for Pharmacovigilance, the Investigator must fulfill his/her obligation according to the law in force in his country.

10.11 General Notes

- In case of death, a comprehensive narrative report of the case should be prepared by the Investigator and sent to the [redacted] Safety Contact together with the Serious Adverse Event form, retaining a copy on site.
- If an autopsy is performed, copy of autopsy report should be actively sought by the Investigator and sent to the [redacted] Safety Contact as soon as available, retaining a copy on site.
- All documents provided by the Investigator or site staff to the [redacted] Safety Contact must be carefully checked for respect of confidentiality. All personal subject's data must be redacted.
- All source documents provided by the Investigator or site staff to the [redacted] Safety Contact must be carefully checked for respect of confidentiality. All personal subjects' data must be redacted.
- In case of pregnancy occurred to the subject's partner, the subject participating to the study should not be discontinued from the study. The pregnancy will be followed with due diligence until the outcome is known and till the age of one year to detect any congenital anomaly or birth defect.
  The pregnancy must be reported by the investigator within 24 hours by fax/e-mail/via Monitor to the [redacted] Safety Contact using the paper Pregnancy Report Form. The [redacted] Safety Contact will inform Chiesi of the pregnancy within one working day of being notified.

The first two pages of the Pregnancy Report Form should be completed by the investigator with all the available information and sent to the [redacted] Safety Contact. The third page will be completed as soon as the investigator has knowledge of the pregnancy outcome, together with a follow-up of the first two pages, if necessary (e.g. an update in the medications received during pregnancy by the mother). If it meets the criteria for immediate classification of a SAE (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital anomaly, birth defect) the Investigator should follow the procedure for reporting SAEs.

- Any Adverse Drug Reaction (ADR) occurring with any marketed non-investigational medicinal product and/or concomitant medication during the study must be reported by the Investigator to his/her concerned Health Authority according to the applicable laws. The Investigator is also recommended to report all adverse drug reactions to the relevant Marketing Authorisation Holders of the involved medicinal products. Additionally, also conditions of use outside the marketing authorisation of the medicinal products (i.e. off-label, overdose, misuse, abuse and medication errors) or from occupational exposure, as well as cases of suspected drug interaction, pregnancy, breast-feeding exposure and lack of efficacy should be reported.

11. DATA MANAGEMENT

An electronic CRF (eCRF) will be filled-in by the Investigator and/or his/her representative designee. Front-end edit checks will run at the time of data collection and back-end edit checks will be used by the Data Manager to check for discrepancies and to ensure consistency and completeness of the data. Medical history, adverse events and concomitant procedures will be coded using the MedDRA dictionary; medications will be coded using the WHO Drug dictionary and Anatomical Therapeutic Chemical classification (ATC) up to level 5.
External data (PK concentrations) will be processed centrally, sent to the designated CRO and reconciled with the corresponding information recorded in the CRF as part of cleaning activities.
Access to electronic systems used for data collection will be granted to the study personnel only after appropriate training.
After the completion of data collection and cleaning, a review meeting will be held to determine the occurrence of any protocol violation and to define the subject populations for the analysis. Once the database has been declared to be complete and accurate, it will be locked, and the planned statistical analysis will be performed.
If the database is unlocked after the initial lock, the process must be carefully controlled and documented; updates to the study data must be authorized by Chiesi.
At the study conclusion, a complete copy of the study data will be created for archival purposes at Chiesi. The investigators will receive copies of the subject data for retention at the investigational sites.

12. STATISTICAL METHODS

12.1 Sample Size

According to the exploratory nature of the study, no formal sample size calculation was performed. A total of 8 subjects will be included in the study to characterize the absolute bioavailability and the mass balance of CHF6001.

12.2 Populations for analysis

- Safety population: **all enrolled subjects who start study treatment inhalation.**
- **PK population:** all subjects from the safety population excluding subjects without any valid PK measurement or with major protocol deviations significantly affecting PK, for example: incorrect inhalation, change in subject condition (cold), failure in delivery of the device, use of non-permitted medications. Exact definition of major protocol deviations concerning PK will be discussed by the clinical team and described in a specific document before bioanalytical data are disclosed. Subjects will not be excluded on the basis of statistical analysis or for pharmacokinetic reasons, except in the cases defined in the EMA "Guideline on the Investigation of Bioequivalence" [21].

PK variables will be analysed in the PK population. The Safety population will be used in the analysis of all safety variables.

12.3 Statistical analysis

A detailed statistical analysis plan will be described in the Statistical Analysis Plan. The plan might be reviewed and updated as a result of the review of the data and will be finalized before locking the database.

12.3.1 Descriptive Statistics

All PK variables (except for t_{max}, t_{max_inh} and t_{max_iv}) will be summarized by means of descriptive statistics including n (number of observed values), arithmetic mean, standard deviation (SD), coefficient of variation (CV), geometric mean, geometric CV, median, minimum and maximum. t_{max}, t_{max_inh}, t_{max_iv} will be summarized by using n, median, minimum and maximum.

General descriptive statistics for other continuous variables will include the n (number of observed values), the mean, the standard deviation, the median, the minimum, and the maximum values.

For categorical variables, the number and percent of subjects with a specific level of the variable will be presented.

12.3.2 Missing data

Details on dealing with missing data, along with the handling of possible outliers, will be described in the SAP.

12.3.3 Subject demographics and baseline characteristics

Demographics and baseline variables will be summarized using descriptive statistics for the Safety population and PK (if different from safety population).

12.3.4 PK variables

The following PK variables will be summarized by means of descriptive statistics as described in Section 12.3.1:

- Plasma [^{14}C]-CHF6001, $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$, V_z, V_{dss} and CL
- Plasma [^{14}C]-total $AUC_{0\text{-}t\ iv}$, C_{max_iv}, t_{max_iv}, $AUC_{0\text{-}\infty\_iv}$, $t_{1/2\ iv}$
- Plasma CHF6001 $AUC_{(0\text{-}t)\_inh}$, C_{max_inh}, t_{max_inh}, $AUC_{(0\text{-}\infty)\_inh}$, $t_{1/2\_inh}$, F_{inh}

- Urine and feces [^{14}C]-CHF6001and [^{14}C]-total excreted fraction (Fe_u, Fe_f and Fe_{u+f})
- [^{14}C]-CHF6001 $CL_{blood,}$ CL_R and CL_{R_blood}
- Blood to plasma ratio ($R_{b/p}$) [^{14}C]-CHF6001
- Blood to plasma ratio ($R_{bt/pt}$) [^{14}C]-total
- Fe_{met}
- Metabolites Fr_plasma, Fr_urine, Fr_feces, Fr_urine+feces
- [^{14}C]-CHF6001 E_{h_blood}

Concentration (mass equivalent in mL or mass in mL)/time curves for plasma total [^{14}C], [^{14}C]-CHF6001 and non-radiolabeled CHF6001 will be presented in linear/linear and log/linear scale.
Plots will be presented based on arithmetic means and by subject (all individual plasma concentration/time curves in one graph).

Bar charts based on arithmetic means showing the percentage of urine and feces excreted fraction for total [^{14}C] and [^{14}C]-CHF6001 in the different time intervals will be presented.

Bar charts based on arithmetic means showing percenatages of the relevant metabolites recovered in plasma, urine and feces will be presented.

All individual concentration data (mass equivalent per mL or mass per mL) and PK parameters will be listed. In addition, a listing of the actual sampling times relative to drug inhalation will be provided. Concentrations will be summarized by scheduled sampling time by using n, arithmetic mean, SD, CV, median, minimum and maximum.

12.3.5 Safety variables

Adverse Events
All adverse events starting on or after the time of first study drug inhalation will be classified as treatment emergent adverse event (TEAE). Any adverse events started after the informed consent signature and before the time of first study drug inhalation will be classified as pre-treatment adverse event.
The number of subjects who experienced at least one TEAE, drug-related TEAE, serious TEAE and TEAE leading to study discontinuation will be summarized. Summaries will be presented overall (number and percentage of subjects having at least one event, total number of events) and by system organ class and preferred term (number and percentage of subjects having at least one occurrence of that event).
All adverse events will be listed.
Pre-treatment adverse event will be listed only.

Vital signs
Percentage of subjects with clinical relevant changes from baseline to 2,5 hrs post-dose in SBP and DBP defined as

- Change from pre-dose in DBP > 10 mmHg;
- Change from pre-dose in SBP > 20 mmHg;

will be summarized.

12-lead safety ECG

Percentage of subjects with clinical relevant changes from baseline to 2,5 hrs post-dose in HR defined as

- Change from pre-dose in HR> 20 bpm

will be summarized.

Laboratory data

For quantitative laboratory parameters (chemistry, haematology and fasting glucose). Shift tables from screening with reference to normal ranges will be summarized.
All laboratory data will be listed with abnormal values flagged.

12.3.6 Interim analysis

Not Applicable.

13. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD APPROVAL

The study proposal will be submitted to the Ethics Committee in accordance with the local requirements.
The EC shall give its opinion in writing -clearly identifying the study number, study title and informed consent form approved-, before the clinical trial commences.
A copy of all communications with the EC will be provided to the Sponsor.
The Investigator should provide written reports to the EC annually or more frequently if requested on any changes significantly affecting the conduct of the trial and/or increasing risk to the subjects (according to the requirements of each country).

14. REGULATORY REQUIREMENTS

The study will be notified to the Health Authorities (or authorized by) according to the local legal requirements.
Selection of the subjects will not start before the approval of the Ethics Committee has been obtained and the study notified to Health Authorities (or authorized by).

15. INFORMED CONSENT

Informed consent must be written in a language understandable to the subjects. It is the responsibility of the Investigator to obtain written consent from each subject prior to any study related procedures taking place, by using the latest EC approved version of the document.
Adequate time shall be given to the subject to enquiry the PI about any clarification needed and to consider his or her decision to participate to the trial.
If the subject is unable to read, the informed consent will be obtained in the presence of an impartial witness, e.g., a person independent of the study who will read the informed consent form and the written information for the subject.
Consent must be documented by the subject's dated signature. The signature confirms that the consent is based on information that has been understood. Moreover, the Investigator must sign and date the informed consent form.
Each subject's signed informed consent must be kept on file by the Investigator. One copy must be given to the subject.

16. SOURCE DOCUMENTS/DATA

16.1 Recording of source data

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data recorded in the eCRF derived from source documents should be consistent with the data recorded on the source documents.

16.2 Direct access to source document/data

The Investigators or designated must permit trial-related monitoring, audits, Ethics Committee review or regulatory inspection, providing direct access to source data/documents.

17. STUDY MONITORING

Monitoring will be performed by [redacted] who has been designated by Chiesi.
It is understood that the monitor(s) will contact and visit the Investigator/centre before the study, regularly throughout the study and after the study had been completed, and that they will be permitted to inspect the various study records: case reports form, Investigator study file and source data, provided that subject confidentiality is respected.
The purposes of these visits are:

- to assess the progress of the study;
- to review the compliance with the study protocol;
- to discuss any emergent problem;
- to check the eCRFs for accuracy and completeness;
- to validate the contents of the eCRFs against the source documents;
- to assess the status of drug storage, dispensing and retrieval;
- Prior to each monitoring visit, the Investigator or staff will record all data generated since the last visit on the case report forms. The Investigator and/or study staff will be expected to be available for at least a portion of the monitoring visit to answer questions and to provide any missing information;
- It is possible that the Investigator site may be audited by Sponsor personnel or regulatory national and/or international regulatory agencies during and after the study has been completed.

18. QUALITY ASSURANCE

The R&D Quality Assurance Department of Chiesi may perform an audit at any time according to the Sponsor's Standard Operating Procedures, in order to verify whether the study is being conducted in agreement with Good Clinical Practices and the protocol.

19. INSURANCE AND INDEMNITY

Chiesi holds and will maintain an adequate insurance policy covering damages arising out of Chiesi's sponsored clinical research studies.
Chiesi will indemnify the Investigator and hold him/her harmless for claims for damages arising out of the investigation, in excess of those covered by his/her own professional liability insurance, providing that the drug was administered under his/her or deputy's supervision and in strict accordance with accepted medical practice and with the study protocol.
The Investigator must notify Chiesi immediately upon notice of any claims or lawsuits.

20. CONFIDENTIALITY

All study documents are provided by the Sponsor in confidence to the Investigator and his/her appointed staff. None of this material may be disclosed to any party not directly involved in the study without written permission from Chiesi.
The Investigator must assure the subject's anonymity will be maintained. The Investigator will keep a separate list with at least the initials, the subject's study numbers, names, and addresses and telephone numbers. The Investigator will maintain this for the longest period of time allowed by his/her own institution and, in any case, until further communication from Chiesi.

21. PREMATURE TERMINATION OF THE STUDY

Both the Sponsor and the Investigator reserve the right to terminate the study at any time. Should this be necessary, the procedures for an early termination or temporary halt will be arranged after consultation by all involved parties.
The Sponsor should submit a written notification to the Regulatory Authority concerned and Ethics Committee providing the justification of premature ending or of the temporary halt.

22. CLINICAL STUDY REPORT

The clinical study report, including the statistical and clinical evaluations, shall be prepared and sent to principal Investigator for agreement and signature.
At the end of the trial a summary of the clinical study report will be provided to the Ethics Committees, to the Competent Authority of the EU Member State and to Investigator.

23. RECORD RETENTION

After completion of the study, all documents and data relating to the study will be kept in an orderly manner by the Investigator in a secure study file.

Regulations require that essential documents must be retained for at least two years after the final marketing approval in an ICH region or until two years have elapsed since the formal interruption of the clinical development of the product under study.

It is the responsibility of the Sponsor to inform the Investigator of when these documents can be destroyed. The Investigator must contact Chiesi before destroying any trial-related documentation. In addition, all subjects' medical records and other source documentation will be kept for the maximum time permitted by the institution.

24. PUBLICATION OF RESULTS

Chiesi is entitled to publish and/or present any results of this study at scientific meetings, and to submit the clinical trial data to national and international Regulatory Authorities and, if they fall under the Chiesi commitments on Clinical Trial Transparency, to make them available on www.chiesi.com website.
Chiesi furthermore reserves the right to use such data for industrial purposes.
In the absence of a Study Steering Committee, Investigators will inform Chiesi before using the results of the study for publication or presentation, and agree to provide the Sponsor with a copy of the proposed presentation. Data from individual study sites must not be published separately.
Negative as well as positive results should be published or otherwise made publicly available according to the relevant regulatory requirements.

25. REFERENCES

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APPENDIX 1 - Approval of the protocol by clinical investigator(s)

EVALUATION OF THE ABSOLUTE BIOAVAILABILITY AND MASS BALANCE OF CHF6001 FOLLOWING A SINGLE INHALED DOSE CO-ADMINISTERED WITH AN INTRAVENOUS RADIOLABELLED MICROTRACER DOSE IN HEALTHY VOLUNTEERS

Product:

- Inhalation: CHF6001 800µg/20mg
- Infusion: [^{14}C]-CHF6001 1,85µg/1ml

Pharmaceutical Form:

- Inhalation: Dry power Inhaler
- Infusion: Solution

Approval of Clinical Study Protocol by the Investigator:

I have carefully read this protocol and I agree that it contains all the necessary information required to conduct the study and I agree to conduct it as described.

I understand that this trial will not be initiated without Ethics Committee/Institutional Review Board approvals and that the administrative requirements of the governing body of the institution will be fully complied with.

Informed written consent will be obtained from all participating subjects and appropriately documented, prior to their enrolment in the study.

The undersigned agrees that the trial will be carried out in conformity with the Declaration of Helsinki (as applicable, with attention being drawn to Section concerning freely given consent), ICH E6 Good Clinical Practices and with all the other local laws and regulations relevant to the use of new and approved therapeutic agents in subjects.

Principal Investigator's Name: _____,MB ChB **Centre No. :** _____

_____ _____
Signature Date

Chiesi Farmaceutici S.p.A.
Via Palermo 26/A
43122 Parma - Italy

APPENDIX 2 – RECOMMENDATIONS RELATED TO CONTRACEPTION AND PREGNANCY TESTING IN CLINICAL TRIALS

Definition of women of childbearing potential and of fertile men

For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile.

Definition of women of non-childbearing potential

For the purpose of this document, a woman is considered of non-childbearing potential (WNOCBP), i.e. sterile, when physiologically incapable of becoming pregnant (i.e. post-menopausal or permanently sterile).

- A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
- Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

Definition of fertile men

For the purpose of this document, a man is considered fertile after puberty unless permanently sterile by bilateral orchidectomy.

Birth control methods, which may be considered as highly effective

For the purpose of this guidance, methods that can achieve a failure rate of less than 1% per year when used consistently and correctly are considered as highly effective birth control methods. Such methods include:

- combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
  - ☐ oral
  - ☐ intravaginal
  - ☐ transdermal
- progestogen-only hormonal contraception associated with inhibition of ovulation:
  - ☐ oral
  - ☐ injectable
  - ☐ implantable
- intrauterine device (IUD)
- intrauterine hormone-releasing system (IUS)
- bilateral tubal occlusion
- vasectomised partner 1
- sexual abstinence 2

[1] Vasectomised partner is a highly effective birth control method provided that partner is the sole sexual partner of the WOCBP trial participant and that the vasectomised partner has received medical assessment of the surgical success.

[2] Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject.

Clinical Trial Facilitation Group guidance: Recommendations related to contraception and pregnancy testing in clinical trials. Final version 2014-09-15

APPENDIX 3 – BREAKDOWN OF BLOOD SAMPLES COLLECTION

Detailed Blood Collection Volumes for PK sampling:

TOTAL VOLUME TO BE COLLECTED PER SUBJECT							
	Clinical chemistry & Fasting glucose	Hematology	Serology	Whole Blood AMS	PK	PK-AMS	Metabolite profiling
Screening	1	1	1				
Day -1							
Day 1				4	12	15	15
Day 2					1	1	1
Day 3					1	1	1
Day 4					1	1	1
Day 5					1	1	1
Day 6					1	1	1
Day 7					1	1	1
Day 8					1	1	1
Day 9					1	1	1
Day 10					1	1	1
Day 11	1	1			1	1	1
Follow up							
Total vol (mL) per sample	*3.5*	*4*	*3.5*	*2*	*4*	*5*	*10*
Tot vol (mL)	*7*	*8*	*3.5*	*8*	*88*	*125*	*250*
Total vol (mL) per subject	**489.5**						