CLINICAL STUDY PROTOCOL

STUDY CODE No.: CLI-05993AB6-06

EudraCT number: 2022-003247-97

NCT05875025

OPEN-LABEL, RANDOMISED, CONTROLLED, 2-WAY CROSS-OVER STUDY TO ASSESS THE EFFECT OF MULTIPLE DOSES OF THE NEW HFA-152a PROPELLANT VERSUS THE MARKETED HFA-134a PROPELLANT ON MUCOCILIARY CLEARANCE IN HEALTHY VOLUNTEERS.

MUCOCILIARY CLEARANCE STUDY

The effect of the new HFA-152a propellant on normal lung clearance

Version No.: 1.0

Version Date: 16 February 2023

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

GENERAL INFORMATION

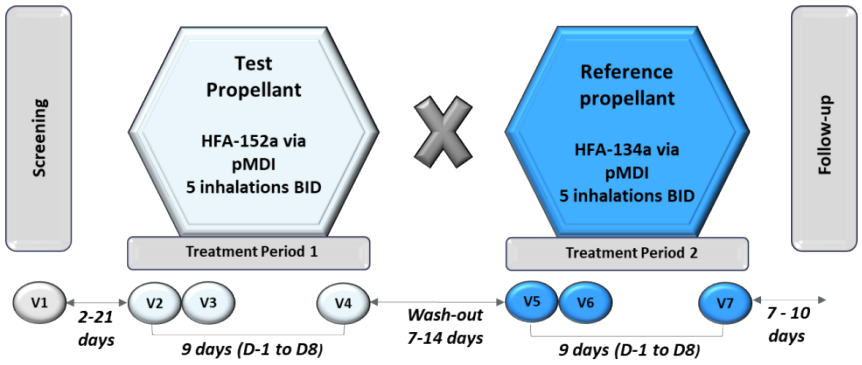
SPONSOR	Chiesi Farmaceutici S.p.A. (hereafter reported also as Chiesi) Via Palermo 26/A 43122 Parma - Italy + 39 0521 2791
SPONSOR'S MEDICAL EXPERT (Clinical Research Physician)	[redacted] Clinical Research Physician Global Clinical Development Tel: [redacted] Mob: [redacted] E-mail: [redacted] Readily available in case of medical questions
INVESTIGATOR	Dr Rory Taylor Principal Investigator Clinical Research Physician CPS Research McCafferty House 99 Firhill Road Glasgow, G20 7BE – United Kingdom
MONITORING CRO	[redacted]

VERSION HISTORY

Version	Date	Change History
1.0	16 February 2023	First version

Clinical Study Code No.: CLI-05993AB6-06	Version No.: 1.0	Date: 16 February 2023
EudraCT number: 2022-003247-97		

PROTOCOL OUTLINE

Study title	Open-label, randomised, controlled, 2-way cross-over study to assess the effect of multiple doses of the new HFA-152a propellant *versus* the marketed HFA-134a propellant on mucociliary clearance in healthy volunteers.
Sponsor	Chiesi Farmaceutici S.p.A. - Via Palermo 26/A 43122 Parma - Italy
Name of the product	Hydrofluoroalkane (HFA)-152a propellant via pressurised metered dose inhaler (pMDI).
Site	Single site, in the United Kingdom.
Indication	Not applicable.
Study design	Multiple-dose, open-label, randomised, controlled, 2-way cross-over study.  (BID=Twice daily; D=Day; HFA=Hydrofluoroalkane; pMDI=Pressurised metered dose inhaler; V=Visit) *The study design proposed in the figure is just an example of one of the possible sequences.*
Study phase	Phase I
Objectives	**Primary objective:** • To assess the effect of multiple doses of the HFA-152a propellant and the HFA-134a propellant (when administered not in combination with active compounds) on mucociliary clearance (MCC). **Secondary objective:** • To evaluate the general safety and tolerability of the two different propellants.
Treatment duration	Two treatment periods, each including an 8-day treatment administration. The two treatment periods will be separated by a 7- to 14-day wash-out period.

Test product dose/route/regimen	**Placebo formulated with HFA-152a propellant via pMDI:** **Administration**: 5 inhalations twice daily (BID [morning and evening]) for 8 consecutive days, starting from the morning of Day 1 until the morning of Day 8.
Reference product dose/route/regimen	**Placebo formulated with HFA-134a propellant via pMDI:** **Administration**: 5 inhalations BID (morning and evening) for 8 consecutive days, starting from the morning of Day 1 until the morning of Day 8.
Number of subjects	20 randomised subjects in order to have at least 18 evaluable subjects.
Study population	Male and female adult healthy volunteers.
Inclusion/exclusion 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. Healthy male and female subjects aged 18-55 years (inclusive); 3. Ability to understand the study procedures, the risks involved and ability to be trained to use the inhalers correctly; 4. Body Mass Index (BMI) between 18 and 30 kg/m^2 (extremes inclusive); 5. Non-smokers or ex-smokers who smoked < 5 pack-years (pack-years = the number of cigarette packs per day, times the number of years) and stopped smoking > 5 years 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 randomisation; 7. Vital signs within normal limits at screening: diastolic blood pressure (DBP) 40-89 mmHg, systolic blood pressure (SBP) 90-139 mmHg, extremes included (two measures performed after at least 5 minutes of resting, the mean value must be within the defined range); body temperature < 37.5°C; *Note: In case of abnormal vital signs at screening, the assessment can be repeated once before randomisation.* 8. 12-lead digitised electrocardiogram (ECG) in triplicate considered as normal at screening: 40 ≤ heart rate (HR) ≤ 110 bpm, 120 ≤ PR interval (PR) ≤ 210 ms, QRS interval (QRS) ≤ 120 ms, and QT interval corrected using Fridericia's formula (QTcF) ≤ 450 ms for males and QTcF ≤ 470 ms for females. The mean value must be within the defined range; *Note: In case of abnormal ECG at screening, the assessment can be repeated once before randomisation.*

	9. Lung function measurements within normal limits at screening: forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) > 0.70 and $FEV_1 \geq 80\%$ predicted; *Note: In case of abnormal lung function at screening, the assessment can be repeated once before randomisation.* 10. Female subjects fulfilling one of the following criteria: a. Women of non-childbearing potential (WONCBP) defined as physiologically incapable of becoming pregnant (i.e. post-menopausal or permanently sterile, as per definitions given in Section 4.1 of the Clinical Trials Facilitation and Coordination Group guidance). Tubal ligation or partial surgical interventions are not acceptable. If indicated, as per Investigator's request, post-menopausal status may be confirmed by follicle-stimulating hormone levels (according to local laboratory ranges); b. Women of childbearing potential (WOCBP) fulfilling one of the following criteria: i. WOCBP with fertile male partners: they and/or their partner must be willing to use a highly effective birth control method preferably with low user dependency, from the signature of the informed consent form (ICF) and until the follow-up call or; ii. WOCBP with non-fertile male partners (contraception is not required in this case). *Note: In case of hormonal contraception, an additional non-hormonal method (barrier method, preferably male condom) is required.* 11. Male subjects fulfilling one of the following criteria: a. Fertile male subjects with a pregnant or non-pregnant WOCBP partner: they must be willing to use male condom, from the signature of the ICF until the follow-up call or; b. Non-fertile male subjects (contraception is not required in this case) or; c. Fertile male subjects with a WONCBP partner (contraception is not required in this case). For the definition of WOCBP, fertile men and the list of highly effective birth control methods, refer to Section 4.1 of the Clinical Trials Facilitation and Coordination Group guidance. *Inclusion criterion #6 will be re-checked before randomisation.*
	The presence of any of the following exclusion criteria will exclude a subject from study enrolment: 1. Participation in another clinical study with an investigational drug in the 3 months or five half-lives of that investigational

	drug (whichever is longer) preceding the administration of the study treatment; a longer and more appropriate time could be considered by the 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, gastrointestinal, renal, endocrine, metabolic, neurologic, or psychiatric disorders that may interfere with successful completion of the study (as described in this study protocol), according to the Investigator's judgment; 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 the Investigator's judgment; *Note: In case of abnormal laboratory values that could indicate a temporary condition, the test can be performed again once before randomisation.* 4. Subjects with history of breathing problems (i.e. history of asthma including childhood asthma); 5. Positive serology test for human immunodeficiency virus (HIV) 1 or HIV2 serology at screening; 6. Positive results from the hepatitis serology, indicating acute or chronic hepatitis B or hepatitis C at screening (e.g. positive hepatitis B surface antigen (HBsAg), immunoglobulin M antibody to hepatitis B core [IgM anti-HBc] antigen, hepatitis C virus [HCV] antibody with detectable HCV ribonucleic acid [RNA]); 7. Blood donation or blood loss (≥ 450 mL) during the 2 months prior to screening or randomisation; 8. Positive urine test for cotinine at screening or prior to randomisation; *Note: In case of a positive test, the test can be repeated once before randomisation*. 9. Documented history of alcohol abuse within 12 months prior to screening, an average weekly alcohol intake of greater than 14 units, or a positive alcohol breath test at screening or prior to randomisation; *Note: In case of a positive test, the test can be repeated once before randomisation*. 10. Documented history of drug abuse within 12 months prior to screening or a positive urine drug screen evaluated at screening or prior to randomisation; *Note: In case of a positive test, the test can be repeated once before randomisation*.

	11. Intake of non-permitted concomitant medications in the predefined period prior to screening or prior to randomisation, or subject expected to take non-permitted concomitant medications during the study; 12. Presence of any current infection, or previous infection that resolved less than 1 week prior to screening or to randomisation; 13. Known intolerance and/or hypersensitivity to any of the excipients contained in the formulation used in the study; 14. Documented coronavirus disease 2019 (COVID-19) diagnosis within the last 2 weeks, or associated complications/symptoms which have not resolved within 2 weeks prior to screening or prior to randomisation; 15. Heavy caffeine drinker (average of > 5 cups or glasses of caffeinated beverages [e.g. coffee, tea, cola], calculated by the number of standard espresso portions, per day); 16. For females only: pregnant or lactating women, where pregnancy is defined as the state of a female after conception and until termination of the gestation, confirmed by a positive serum human chorionic gonadotropin laboratory test; *Note: A serum pregnancy test will be performed at screening only. A urine pregnancy test will be collected on Day -1 of each treatment period.* 17. The use of any kind of smoking electronic devices (e.g. e-cigarettes), within 6 months before screening or prior to randomisation; 18. Subjects for whom participation in this study will exceed a total radiation exposure of 5 mSv in the last 12-month period or 10 mSv in the last 5-year period; 19. Subjects with a total dosimetry value (including history of exposure through occupation) which, according to the Investigator's or a medically qualified designee's judgement, contraindicates their participation in the study. *The following criteria will be re-checked before randomisation: #7, #8, #9, #10, #11, #12, #14, #16 and #17.*
Study plan	A total of 7 ambulatory study visits (Visit 1 [V1] to Visit 7 [V7]) and a follow-up call will be performed during the study, as follows: • A **screening visit** (**V1**) will be carried-out 2 to 21 days before Treatment Period 1 (TP1), to assess subjects' eligibility. • The **Investigational Phase** will comprise two 9-day treatment periods, separated by 7 to 14 days of wash-out from the propellant. Each treatment period will consist of 3 ambulatory visits at the investigational site.

	Treatment Period 1 (TP1) will consist of: o **Visit 2 (V2)** (Day -1 of TP1) including the baseline MCC assessments; o **Visit 3 (V3)** (morning of Day 1 of TP1) including randomisation, the administration of the first dose of the assigned study treatment (HFA-152a or HFA-134a; according to each subject's randomisation arm), and the performance of the planned assessments; *Between V3 and V4, subjects will continue the study treatment administration at home, and will use daily record cards (DRCs) to record compliance to treatment.* o **Visit 4 (V4)** (morning of Day 8 of TP1) including the administration of the last dose of study medication assigned during TP1, and the assessment of its effect by performing the planned assessments (including the post-treatment MCC assessments). **Treatment Period 2 (TP2)** will consist of: o **Visit 5 (V5)** (Day -1 of TP2) including a repetition of the baseline MCC assessments; o **Visit 6 (V6)** (morning of Day 1 of TP2) including the administration of the first dose of the other study treatment, and the performance of the planned assessments; *Between V6 and V7, subjects will continue the study treatment administration at home, and will use DRCs to record compliance to treatment.* o **Visit 7 (V7)** (morning of Day 8 of TP2) including the administration of the last dose of the other study medication, and the assessment of its effect by performing the planned assessments (including the post-treatment MCC assessments). • A **follow-up call** (or visit, if deemed necessary by the Investigator) will be performed between 7 and 10 days from the last intake of the study treatment (also in cases of premature discontinuation, if the early termination visit is performed less than 7 days after the last dose of study treatment). **Measures** The following measures will be evaluated at each treatment period (TP1 and TP2): • MCC: image acquisitions for the assessment of MCC will be done at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of the radiotracer, on Day -1 and on Day 8 of each treatment period;

	Note: On Day 8, the radiotracer will be administered at post-dose, approximately 2 hours after administration of the last dose of the propellant. • Spirometry: FEV_1 and FVC will be measured at pre-dose (within 30 minutes before) and at post-dose (20 minutes after) of the morning administration of the propellant, on Day 1 and Day 8 of each treatment period, and before the inhalation of radiolabelled particles; • Vital signs (SBP, DBP in duplicate and oxygen saturation levels [SpO_2], in single) and local 12-lead safety ECG measurements (in single) will be evaluated at pre-dose (within 1 hour before the intake of the first inhalation of the propellant) and at post-dose (5 minutes, 15 minutes, and 1 hour 45 minutes after the last inhalation of the propellant) of the morning administration of the propellant, on Day 1 and Day 8 of each treatment period.
Most relevant allowed concomitant treatments	• Occasional paracetamol (maximum 2 g per day, with a maximum of 10 g per 14 days, for mild non-excluding conditions [see exclusion criterion #2]); • Hormonal contraceptives; • Hormonal replacement treatment for post-menopausal women; • COVID-19 vaccine during the wash-out periods and at least 7 days before Day 1 of each treatment period. *Note: Any systemic symptoms (e.g. myalgia, fever, chills, fatigue) after COVID-19 vaccine should subside 2 days before the next treatment period. Otherwise, wash-out period extension should be evaluated on a case-by-case basis.*
Most relevant forbidden concomitant treatments	• From 3 months before screening and up-to the end of the follow-up period: 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); • From 3 weeks before randomisation and up-to the end of the follow-up period: any drug treatment, including prescribed or over-the-counter medicines, vitamins, herbal medicines, homeopathic remedies, etc., with the exception of the allowed concomitant medications specified above.
Study variables	<u>Study variables evaluating the primary objective (i.e. to assess the effect of multiple doses of each propellant on MCC)</u>: • MCC rate, as assessed by the percent particle retention at 2 hours after the inhalation of radiolabelled particles (PPR_2); • MCC rate, as assessed by the percent particle retention at 4 hours after the inhalation of radiolabelled particles ($PPR_{4)}$.

	Study variable completing the evaluation of the primary objective: • MCC, as assessed by the area under the tracheobronchial particle retention curve between 0 and 4 hours (AUC_{0-4}) after the inhalation of radiolabelled particles.
Safety variables	Variables evaluating the secondary objective (i.e. to evaluate the general safety and tolerability of the two different propellants): • Adverse events (AEs); • Vital signs (SBP, DBP and SpO_2); • 12-lead ECG parameters: HR, QRS, PR and QTcF. • Lung function parameters: FEV_1 and FVC.
Sample size calculation	No formal sample size calculation has been performed for this study, considering its exploratory nature. A total of 20 subjects will be randomised in the study in order to reach at least 18 evaluable subjects.
Statistical methods	**Study variables** MCC variables (in whole lung) evaluating the primary objective: • The change from baseline in PPR_2 at Day 8 will be analysed using an analysis of covariance (ANCOVA) model, including treatment, subject and period as fixed effects, and PPR_2 at Day -1 as a covariate. The adjusted mean change from baseline obtained with each treatment and the adjusted mean difference between HFA-152a and HFA-134a with their 95% confidence intervals (CIs) and p-values will be estimated by the model; • The change from baseline in PPR_4 at Day 8 will be analysed using the same model used for the change from baseline in PPR_2. MCC variable completing the evaluation of the primary objective: • The change from baseline in AUC_{0-4} after the inhalation of radiolabelled particles will be summarised using descriptive statistics. **Safety variables** • The number of treatment-emergent adverse events (TEAEs) and the number and percentage of subjects who experienced at least one TEAE will be presented for each study treatment for all AEs, adverse drug reactions (ADRs), serious AEs, non-serious AEs, serious ADRs, severe AEs, AEs leading to study discontinuation, and AEs leading to death. Summaries will be presented overall and by system organ class (SOC) and preferred term (PT) based on the Medical Dictionary for Regulatory Activities (MedDRA). • For 12-lead ECG parameters (HR, PR, QRS, QTcF) and vital signs (SBP, DBP and SpO_2), the mean absolute value with its 95% CI and the mean change from pre-dose with its 90% CI

	(95% CI for vital signs) will be presented by treatment at each post-dose timepoint. • Descriptive statistics on actual values and change from baseline will be presented for FEV_1 and FVC.

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

99mTc	Technetium-99m
ADR	Adverse Drug Reaction
AE	Adverse Event
ALT	Alanine Aminotransferase
ANCOVA	Analysis of Covariance
ARSAC	Administration of Radioactive Substances Advisory Committee
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
AUC_{0-4}	Area Under the Tracheobronchial Particle Retention Curve Between 0 and 4 hours
BID	Twice Daily
CI	Confidence Interval
CIOMS	Council for International Organizations of Medical Sciences
Co-57	57-Cobalt
COPD	Chronic Obstructive Pulmonary Disease
COVID-19	Coronavirus Disease 2019
CRO	Contract Research Organisation
CS	Clinically Significant
CSR	Clinical Study Report
DALY	Disability Adjusted Life Year
DBP	Diastolic Blood Pressure
DPO	Data Protection Officer
DRC	Daily Record Card
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
FEV_1	Forced Expiratory Volume in 1 Second
FSH	Follicle-Stimulating Hormone
FVC	Forced Vital Capacity
γ-GT	Gamma-Glutamyl Transpeptidase
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation
GWP	Global Warming Potential
Hb	Haemoglobin
HBsAg	Hepatitis B Surface Antigen
Hct	Haematocrit
HCV	Hepatitis C Virus
HFA	Hydrofluoroalkane
HIV	Human Immunodeficiency Virus

HR	Heart Rate
ICF	Informed Consent Form
ICH	International Council for Harmonisation Technical Requirements for Pharmaceuticals for Human Use
ICSR	Individual Case Study Report
IgM anti-HBc	Immunoglobulin M Antibody to Hepatitis B Core
IMP	Investigational Medicinal Product
ITT	Intention-to-Treat
MCC	Mucociliary Clearance
MedDRA	Medical Dictionary for Regulatory Activities
MHRA	Medicines and Healthcare Products Regulatory Agency
MRT	Mean Residence Time
OIP	Orally Inhaled Products
pH	Potential of Hydrogen
PK	Pharmacokinetic
PLT	Platelet
pMDI	Pressurised Metered Dose Inhaler
PP	Per-Protocol
PPR	Percent Particle Retention
PPR_2	Percent Particle Retention at 2 hours after the Inhalation of Radiolabelled Particles
PPR_4	Percent Particle Retention at 4 hours after the Inhalation of Radiolabelled Particles
PR	PR Interval
PT	Preferred Term
QRS	QRS Interval
QTcF	QT Interval Corrected Using Fridericia's Formula
RBC	Red Blood Cell
R&D	Research and Development
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SBP	Systolic Blood Pressure
SmPC	Summary of product characteristics
SOC	System Organ Class
SpO_2	Oxygen Saturation Levels
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-Emergent Adverse Event
T_{max}	Time to Maximum Plasma Concentration
TP	Treatment Period
UK	United Kingdom
V	Visit
WBC	White Blood Cell

WHO	World Health Organization
WOCBP	Women of Childbearing Potential
WONCBP	Women of Non-Childbearing Potential

CONTENT

1. INTRODUCTION

1.1 BACKGROUND INFORMATION

Chronic respiratory diseases

Chronic respiratory diseases are among the leading causes of morbidity and mortality worldwide [1]. Among these, common chronic respiratory diseases include chronic obstructive pulmonary disease (COPD) with a global prevalence of 3.9% in 2017, and asthma with a global prevalence of 3.6% in 2017 [2].

COPD is a chronic inflammatory disease of the airways characterised by persistent respiratory symptoms (e.g. cough, breathlessness, excess sputum production) and airway limitation, with a progressive decline in lung function. COPD has seen a recent increase in prevalence, and represents a substantial and increasing economic and social burden [3], [4].

Asthma is a serious, sometimes fatal disease of airways affecting people of all ages. Asthma is characterised by chronic airway inflammation, respiratory symptoms (e.g. wheeze, shortness of breath, cough, chest tightness), and variable expiratory flow limitation. The number of disability adjusted life years (DALYs) lost due to asthma amounts to 26.2 million, representing about 1% of DALYs lost due to any disease, and is comparable to those lost due to diabetes or Alzheimer's disease [5].

COPD and asthma treatments help dilate major air passages, improve shortness of breath and quality of life, and prevent disease escalation and exacerbations [2].

Use of propellants in inhaler devices

Pressurised metered dose inhalers (pMDIs) represent one of the most commonly used devices for inhaled drug delivery in the treatment of COPD and asthma. The propellant, a liquified gas, provides the force required to generate the aerosol cloud.

More than 630 million pMDIs are estimated to be manufactured annually worldwide using up-to 10,000 tons of propellants [6]. The adoption of the Montreal Protocol in 1989 initiated the development of the hydrofluoroalkane (HFA) propellants currently used in pMDIs (HFA-134a and HFA-227ea), which replaced ozone depleting substances [7]. However, there is growing concern regarding the global warming potential (GWP) of many hydrofluorocarbons, including HFA-134a. This led to the introduction of regulations in many parts of the world that place controls on the usage of these substances. Accordingly, manufacturers of aerosolised products have been looking for potential alternatives to these HFAs in both industrial and medical application sectors. The transition towards a low GWP propellant is also in line with Chiesi's sustainability commitment.

A potential alternative to fluorocarbons with high ozone depleting potential and/or GWP in the pMDI application is HFA-152a (1,1 difluoroethane), designated by the United States Environmental Protection Agency as an acceptable replacement in certain applications, including as an aerosol propellant. HFA-152a has an environmental GWP one order of magnitude lower than HFA-134a or HFA-227ea, and a carbon footprint that is 90% reduced compared to an equivalent HFA-134a pMDI [8], [9], [10].

The toxicological properties of HFA-152a are well-known in industrial applications and indicate a relatively low risk profile. However, additional data proving its safety for use as an inhaled drug are required before it can be approved as a novel excipient.

In non-clinical studies, the dose limiting effects of HFA-152a are associated with concentrations far greater than that delivered by a pMDI formulation, and the effects appear to resolve quickly following cessation of exposure [11]. HFA-152a essentially caused no other toxicities.

The pharmacokinetics (PK) of HFA-152a has been studied in healthy volunteers exposed to 0, 200 or 1000 ppm of HFA-152a for 2 hours during light exercise in an exposure chamber [12]. Symptom ratings and changes in inflammatory markers revealed no exposure-related effects.

A first-in-human study assessing the safety, tolerability, taste tolerance, lung function, and blood PK of inhaled HFA-152a (administered alone, 4 puffs via pMDI) in 8 healthy volunteers has been recently completed (study run by Koura UK Ltd) [13]. Each puff contained a maximum of 50 µL of HFA-152a, for a total volume of 200 µL at each administration. The study showed that administration of HFA-152a via pMDI was safe and well-tolerated, with no impact on lung function, vital signs and laboratory values, no adverse events (AEs), and a minimal impact on taste. The PK analysis performed on the HFA-152a concentration in whole blood *versus* time showed rapid absorption, with all participants (except one) having their time to maximum plasma concentration (t_{max}) as the first assayed timepoint collected immediately after dosing. The elimination half-life was not evaluable, since the propellant was eliminated very rapidly from blood and there were too few timepoints in the terminal elimination phase. In order to provide information about the clearance of the propellant, the mean residence time (MRT) was determined. The average MRT was about 9 minutes, which confirmed a rapid clearance from the body.

A study comparing the potential for bronchoconstriction and the general safety and tolerability of the HFA-152a propellant (administered not in combination with active compounds) to those of the HFA-134a propellant (administered not in combination with active compounds) in subjects with mild asthma is ongoing, and studies assessing the lung availability and systemic exposure of drugs delivered throughout the HFA-152a propellant are planned. These studies aim to provide evidence applicable to all the products under development with HFA-152a by Chiesi (Trimbow®, Foster® and Clenil®, all strengths under development).

This study aims to assess the effect on mucociliary clearance (MCC) of multiple doses of the HFA-152a propellant and the HFA-134a propellant (when administered not in combination with active compounds) in adult healthy volunteers.

1.2 STUDY RATIONALE

This clinical study has been designed to investigate the effect on MCC of the new HFA-152a propellant in comparison with the marketed HFA-134a propellant, by measuring the clearance from the lungs of inhaled radioaerosol.

This study was conceived in accordance with the guidelines on orally inhaled products (OIP), specifying that "it may be necessary to assess any effect that the new propellant or excipient may have on mucociliary clearance" [14].

MCC is an effective mechanism operating in the lungs to clear the airways of mucus, of inhaled infectious and toxic particles, and of locally-produced biological debris [15]. This mechanism consists of secretory and ciliated cells that beat with coordination and generate a propulsive force, which mobilises the mucus blanket toward the larynx for elimination [16]. MCC is affected by a variety of factors, including lung diseases and pharmacologic agents [17]. Its deterioration is associated with a reduction in the protective role of the ciliated epithelium and, in such cases, cough and adequate airflow become critical mechanisms for airway clearance [16].

Considering that pMDI devices represent one of the most commonly used devices for inhaled drug delivery in the treatment of chronic respiratory diseases, it was considered important to investigate the effect on MCC of the new HFA-152a propellant in comparison with the marketed HFA-134a propellant. As this residual risk was deemed not adequately covered by the clinical development

program, and as it cannot be predicted by *in-vitro* data, this appropriately designed clinical study will adequately address this residual risk.

This open-label, randomised, controlled, 2-way cross-over study will be conducted in male and female adult (aged 18 to 55 years old [inclusive]) healthy volunteers.

Each subject will be administered multiple doses (5 inhalations twice daily [BID] for 8 consecutive days) of each of the two following treatments, according to an order pre-specified by the randomisation list:

- **Test treatment**: placebo HFA-152a propellant via pMDI;
- **Reference treatment**: placebo HFA-134a propellant via pMDI.

The selected treatment dose (i.e. 5 inhalations BID) corresponds to the maximum total daily dose of HFA-134a propellant allowed for administration with the Clenil pMDI device, according to the current summary of product characteristics (SmPC) (for Clenil 100 µg, the maximum allowed daily dose is 1000 µg, administered as 500 µg BID).

The effect on MCC of each propellant will be evaluated by the change, from baseline (before administration of each propellant) to the end of the 8-day treatment period, in the percent particle retention (PPR) evaluated at 0 (immediately), 2 and 4 hours after the administration of a radiotracer.

1.3 RISK/BENEFIT ASSESSMENT

1.3.1 RISK EVALUATION

The toxicological properties of HFA-152a are well-known in industrial applications and indicate a relatively low risk profile. Short-term inhalation of HFA-152a with light exercise did not have exposure effects in human subjects [12]. A comprehensive package of toxicology studies was conducted for the HFA-152a propellant alone, in conformance with the International Council for Harmonisation Technical Requirements for Pharmaceuticals for Human Use (ICH) M3(R2) requirements; results were described in the Investigator's Brochure for CHF 5993 pMDI HFA-152a. The pre-clinical studies did not elicit any findings that could not be attributed to the expected pharmacological action of the test items, and were similar to those previously reported with the formulation of CHF 5993 HFA-134a propellant. It was therefore considered that the change of the propellant to HFA-152a had not produced any new effects. Of particular note, the results of bacterial mutagenicity, *in-vitro* micronucleus, and *in-vivo* micronucleus assays did not show HFA-152a to be mutagenic or genotoxic. However, the results of the embryofoetal toxicology study in rats were not available at the time of the study. Thus, considering the recommendations related to contraception and pregnancy testing in clinical trials from the Clinical Trial Facilitation and Coordination Group [18], the worst-case scenario was considered, i.e. "Demonstrated or suspected human teratogenicity/fetotoxicity in early pregnancy" and contraception measures were taken accordingly.

This study will involve the subjects' exposure to ionising radiation, due to the administration of a radiotracer and the performance of a transmission scan (see Section 7.2.1). Long-term exposure (years or decades) to ionising radiation can cause cancer. The risk of developing cancer as a consequence of taking part in this study is less than 0.01%, which is considered as negligible risk (for comparison, in the general population, the natural lifetime cancer incidence is around 50%) [19], [20]. The radiation dosimetry for this study is summarised in Table 1 below. The radiotracer technetium-99m (99mTc) albumin colloid will be used in this study. Each subject will receive a maximum of 4 administrations of 99mTc-albumin throughout the course of the study, with a target dose of 1-2 MBq per administration (see Table 1). Transmission scan will be performed once during the study, using a planar 57-Cobalt (Co-57) sheet source which has a total effective dose of 0.07 mSv

per scan. The radiation dose to be administered in this study has been reviewed by the appointed Medical Physics Expert and Clinical Radiation Expert, in line with current regulations. As a precautionary measure, subjects with a historical exposure (5 mSv in the last 12 months or 10 mSv in the last 5 years) or a total dosimetry value which contraindicates their participation will not be allowed in this study (see Section 4.3, exclusion criteria #18 and #19). Each subject will receive a maximum total ionising radiation exposure of 0.27 mSv. This is equivalent to approximately 1 month of average natural background radiation dose in the United Kingdom (UK) or around 14 chest posteroanterior x-ray examinations.

Table 1. Radiation dosimetry of the study

Procedure	Radioactivity per administration	Number of administration	A Total Dose (MBq)	B Effective Dose per administration (mSv)	Total Effective Dose (A x B)
Co-57 transmission scan	-	1	-	-	0.07 mSv
Inhalation of 99mTc- albumin colloid	1-2 MBq	4	8 MBq (maximum 2 MBq per administration)	0.05 mSv	0.2 mSv
99mTC= Technetium-99m; Co-57=57-Cobalt.					

The study will be conducted in healthy volunteers. The study population is free of ciliary dysfunction and free of any inhaled medications, and therefore is considered as sensitive for the assessment of the impact of the new HFA-152a propellant on MCC function. Moreover, MCC has been shown to be altered in (healthy) smokers [21],[22]. Therefore, only non-smoker subjects will be included. The subjects will be closely monitored and evaluated by the Investigator during the entire duration of the study. Therefore, the overall risk/benefit assessment for the proposed study is considered to be acceptable.

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

1.3.2 BENEFIT EVALUATION

This is a MCC study during which healthy subjects will be administered placebo propellants. Therefore, participation in this study does not imply any benefits for the subjects.

1.3.3 CONCLUSIONS

Considering the safety profile of the investigational medicinal products (IMPs), the minimal radiation risk to subjects, the measures in place to assure the subjects' safety and the expected scientific value, the overall risk/benefit assessment can be considered acceptable for the proposed study.

2. STUDY OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To assess the effect of multiple doses of the HFA-152a propellant and the HFA-134a propellant (when administered not in combination with active compounds) on MCC.

2.2 SECONDARY OBJECTIVE

- To evaluate the general safety and tolerability of the two different propellants.

2.3 EXPLORATORY OBJECTIVE

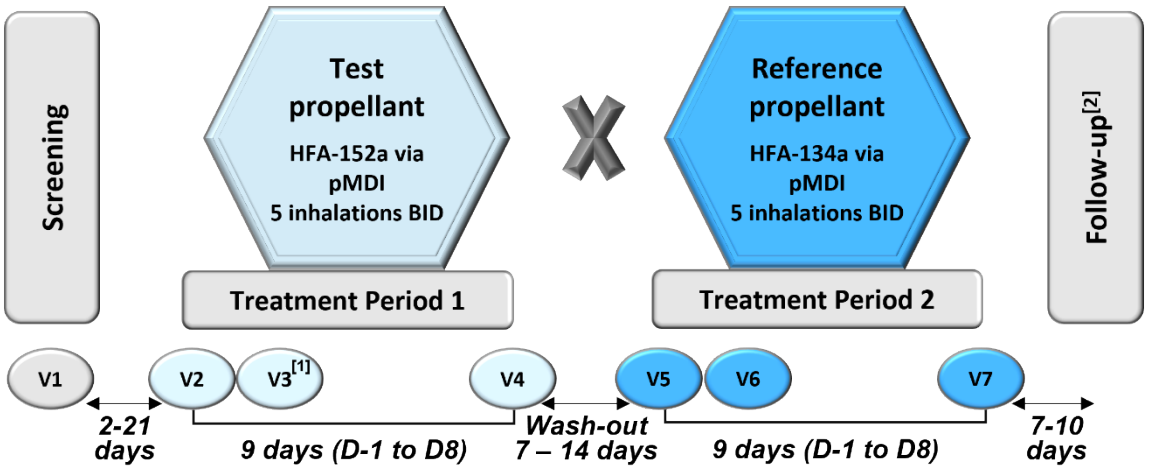
Not applicable.

3. STUDY DESIGN

This is a Phase I, single-centre, multiple-dose, randomised, open-label, controlled, 2-way cross-over study to assess the effect on MCC of the new HFA-152a propellant (5 inhalations BID for 8 days) *versus* the marketed HFA-134a propellant (5 inhalations BID for 8 days) in adult (aged 18 to 55 years old [inclusive]) healthy volunteers.

The study design is shown in Figure 1.

Figure 1. Study design



BID=Twice daily; D=Day; HFA=Hydrofluoroalkane; pMDI=Pressurised metered dose inhaler; V=Visit.

The study design proposed in the figure is just an example of one of the possible sequences.

[1] Randomisation performed at this visit.

[2] A follow-up visit (instead of a call) can be performed, if deemed necessary by the Investigator.

Study treatment

Approximately 20 subjects fulfilling the study eligibility criteria (see Section 4.2 and Section 4.3) will be randomised to receive multiple doses (5 inhalations BID for 8 days) of each of the following treatments, according to an order determined by the randomisation list:

- **HFA-152a propellant** (also referred to as "test treatment"): placebo HFA-152a propellant (not in combination with other active compounds) via pMDI;
- **HFA-134a propellant** (also referred to as "reference treatment"): placebo HFA-134a propellant (not in combination with other active compounds) via pMDI.

Study visits

A total of 7 ambulatory study visits (Visit 1 [V1] to Visit 7 [V7]) and a follow-up call will be performed during the study, as follows:

- A **screening visit** (**V1**) will be carried-out 2 to 21 days before Treatment Period 1 (TP1), to assess a subject's eligibility.
- The **Investigational Phase** will comprise two 9-day treatment periods, separated by 7 to 14 days of wash-out from the propellant. Each treatment period will consist of 3 ambulatory visits at the investigational site.

  Treatment Period 1 (TP1) will consist of:
  - **Visit 2 (V2)** (Day -1 of TP1) including the baseline MCC assessments;
  - **Visit 3 (V3)** (morning of Day 1 of TP1) including randomisation, the administration of the first dose of the assigned study treatment (HFA-152a or HFA-134a; according to each subject's randomisation arm), and the performance of the planned assessments;
    Between V3 and V4, subjects will continue the study treatment administration at home, and will use daily record cards (DRCs) to record compliance to treatment.
  - **Visit 4 (V4)** (morning of Day 8 of TP1) including the administration of the last dose of the study medication assigned during TP1, and the assessment of its effect by performing the planned assessments (including the post-treatment MCC assessments).

  Treatment Period 2 (TP2) will consist of:
  - **Visit 5 (V5)** (Day -1 of TP2) including a repetition of the baseline MCC assessments;
  - **Visit 6 (V6)** (morning of Day 1 of TP2) including the administration of the first dose of the other study treatment, and the performance of the planned assessments;
    Between V6 and V7, subjects will continue the study treatment administration at home, and will use DRCs to record compliance to treatment.
  - **Visit 7 (V7)** (morning of Day 8 of TP2) including the administration of the last dose of the other study medication, and the assessment of its effect by performing the planned assessments (including the post-treatment MCC assessments).
- A **follow-up call** (or visit, if deemed necessary by the Investigator) will be performed between 7 and 10 days from the last intake of the study treatment (also in cases of premature discontinuation, if the early termination visit is performed less than 7 days after the last dose of the study treatment). A subject's participation in the study will end with the follow-up call (or visit, if deemed necessary by the Investigator).

The end of the study is defined as the last follow-up contact of the last subject in the study.

4. SUBJECT SELECTION CRITERIA

The Investigator will evaluate each subject on the selection criteria below and will make the final decision on eligibility.

4.1 SUBJECT RECRUITMENT

Male and female healthy volunteers aged 18 to 55 years old (inclusive) will be selected.

A total of 20 subjects will be randomised in accordance with the inclusion and exclusion criteria, in order to reach at least 18 evaluable subjects.

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. Healthy male and female subjects aged 18-55 years (inclusive);
3. Ability to understand the study procedures, the risks involved and ability to be trained to use the inhalers correctly;
4. Body Mass Index (BMI) between 18 and 30 kg/m^2 (extremes inclusive);
5. Non-smokers or ex-smokers who smoked < 5 pack-years (pack-years = the number of cigarette packs per day, times the number of years) and stopped smoking > 5 years 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 randomisation;
7. Vital signs within normal limits at screening: diastolic blood pressure (DBP) 40-89 mmHg, systolic blood pressure (SBP) 90-139 mmHg, extremes included (two measures performed after at least 5 minutes of resting, the mean value must be within the defined range); body temperature < 37.5°C;

   Note: In case of abnormal vital signs at screening, the assessment can be repeated once before randomisation.
8. 12-lead digitised electrocardiogram (ECG) in triplicate considered as normal at screening: 40 ≤ heart rate (HR) ≤ 110 bpm, 120 ≤ PR interval (PR) ≤ 210 ms, QRS interval (QRS) ≤ 120 ms, and QT interval corrected using Fridericia's formula (QTcF) ≤ 450 ms for males and QTcF ≤ 470 ms for females. The mean value must be within the defined range;

   Note: In case of abnormal ECG at screening, the assessment can be repeated once before randomisation.
9. Lung function measurements within normal limits at screening: forced expiratory volume in 1 second (FEV_1)/forced vital capacity (FVC) > 0.70 and FEV_1 ≥ 80% predicted;

   Note: In case of abnormal lung function at screening, the assessment can be repeated once before randomisation.
10. Female subjects fulfilling one of the following criteria:
    a. Women of non-childbearing potential (WONCBP) defined as physiologically incapable of becoming pregnant (i.e. post-menopausal or permanently sterile, as per definitions given in Appendix 2 and in Section 4.1 of the Clinical Trials Facilitation and Coordination Group guidance). Tubal ligation or partial surgical interventions are not acceptable. If indicated, as per Investigator's request, post-menopausal status may be confirmed by follicle-stimulating hormone (FSH) levels (according to local laboratory ranges);

b. Women of childbearing potential (WOCBP) fulfilling one of the following criteria:
   i. WOCBP with fertile male partners: they and/or their partner must be willing to use a highly effective birth control method preferably with low user dependency, from the signature of the informed consent form (ICF) and until the follow-up call or;
   ii. WOCBP with non-fertile male partners (contraception is not required in this case).

Note: In case of hormonal contraception, an additional non-hormonal method (barrier method, preferably male condom) is required.

11. Male subjects fulfilling one of the following criteria:
    a. Fertile male subjects with a pregnant or non-pregnant WOCBP partner: they must be willing to use male condom, from the signature of the ICF until the follow-up call or;
    b. Non-fertile male subjects (contraception is not required in this case) or;
    c. Fertile male subjects with a WONCBP partner (contraception is not required in this case).

    For the definition of WOCBP, fertile men and the list of highly effective birth control methods, refer to Appendix 2 or to Section 4.1 of the Clinical Trials Facilitation and Coordination Group guidance.

Inclusion criterion #6 will be re-checked before randomisation.

4.3 EXCLUSION CRITERIA

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

1. Participation in another clinical study with an investigational drug in the 3 months or five half-lives of that investigational drug (whichever is longer) preceding the administration of the study treatment; a longer and more appropriate time could be considered by the 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, gastrointestinal, renal, endocrine, metabolic, neurologic, or psychiatric disorders that may interfere with successful completion of the study (as described in this study protocol), according to the Investigator's judgment;
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 the Investigator's judgment;

   Note: In case of abnormal laboratory values that could indicate a temporary condition, the test can be performed again once before randomisation.
4. Subjects with history of breathing problems (i.e. history of asthma including childhood asthma);
5. Positive serology test for human immunodeficiency virus (HIV) 1 or HIV2 serology at screening;
6. Positive results from the hepatitis serology, indicating acute or chronic hepatitis B or hepatitis C at screening (e.g. positive hepatitis B surface antigen [HBsAg], immunoglobulin M antibody to hepatitis B core [IgM anti-HBc] antigen, hepatitis C virus [HCV] antibody with detectable HCV ribonucleic acid [RNA]);
7. Blood donation or blood loss (≥ 450 mL) during the 2 months prior to screening or randomisation;

8. Positive urine test for cotinine at screening or prior to randomisation;
   Note: In case of a positive test, the test can be repeated once before randomisation.
9. Documented history of alcohol abuse within 12 months prior to screening, an average weekly alcohol intake of greater than 14 units, or a positive alcohol breath test at screening or prior to randomisation;
   Note: In case of a positive test, the test can be repeated once before randomisation.
10. Documented history of drug abuse within 12 months prior to screening or a positive urine drug screen evaluated at screening or prior to randomisation;
    Note: In case of a positive test, the test can be repeated once before randomisation.
11. Intake of non-permitted concomitant medications in the predefined period prior to screening or prior to randomisation, or subject expected to take non-permitted concomitant medications during the study;
12. Presence of any current infection, or previous infection that resolved less than 1 week prior to screening or to randomisation;
13. Known intolerance and/or hypersensitivity to any of the excipients contained in the formulation used in the study;
14. Documented coronavirus disease 2019 (COVID-19) diagnosis within the last 2 weeks, or associated complications/symptoms which have not resolved within 2 weeks prior to screening or prior to randomisation;
15. Heavy caffeine drinker (average of > 5 cups or glasses of caffeinated beverages [e.g. coffee, tea, cola], calculated by the number of standard espresso portions, per day);
16. For females only: pregnant or lactating women, where pregnancy is defined as the state of a female after conception and until termination of the gestation, confirmed by a positive serum human chorionic gonadotropin laboratory test;
    Note: A serum pregnancy test will be performed at screening only. A urine pregnancy test will be collected on Day -1 of each treatment period.
17. The use of any kind of smoking electronic devices (e.g. e-cigarettes), within 6 months before screening or prior to randomisation;
18. Subjects for whom participation in this study will exceed a total radiation exposure of 5 mSv in the last 12-month period or 10 mSv in the last 5-year period;
19. Subjects with a total dosimetry value (including history of exposure through occupation) which, according to the Investigator's or a medically qualified designee's judgement, contraindicates their participation in the study.

The following criteria will be re-checked before randomisation: #7, #8, #9, #10, #11, #12, #14, #16 and #17.

4.4 STUDY RESTRICTIONS

The following restrictions should be applied during the study and should be checked at the clinical unit at each visit, from the start of the screening visit and until the end of the last treatment period (i.e. when the subject leaves the clinic on Day 8 of TP2):

- No smoking or intake of substances containing nicotine, and no use of any kind of smoking electronic device (e.g. e cigarettes): during the entire study period (excluding the follow-up).

The following restriction should be applied before the screening visit:

- No strenuous activities: within 24 hours before the start of the screening visit and until the end of the visit.

The following restrictions should be applied prior to each visit with spirometry assessments (i.e. screening visit and Day 1 and Day 8 of both TP1 and TP2) and before each visit with MCC assessments (i.e. Day -1 and Day 8 of both TP1 and TP2):

- No intake of intoxicants such as alcohol, caffeine/xanthine containing beverages or food (coffee, tea, chocolate, cola) within 8 hours. Decaffeinated products are permitted;
- No strenuous activities within 1 hour;
- Avoiding wearing clothes that substantially restrict full chest and abdominal expansion.

The following restriction should be applied prior to each visit with blood collection for laboratory tests (i.e. the screening visit and Day 8 of TP2, or the early termination visit [if applicable]):

- No food intake: within 10 hours before the blood collection.

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

4.5 DISCONTINUATION FROM STUDY

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

- An AE 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 a violation of inclusion or exclusion criteria;
- The subject's safety is affected by the use of a non-permitted concomitant medication;
- The subject, in the opinion of the Investigator, is unwilling or unable to adhere to the study requirements (i.e. non-compliance);
- The Sponsor, the regulatory authorities, or the Ethics Committee(s) (EC[s]), for any reason, terminates the entire study or for this particular subject;
- The subject becomes pregnant.

It is understood by all concerned that an excessive rate of discontinuations can render a study uninterpretable; therefore, unnecessary discontinuations 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.

If a subject withdraws consent and cannot or is unwilling to attend the early termination visit, this visit will not be carried-out and no data will be collected after withdrawal.

If the subject has not withdrawn his/her consent, an early termination visit should be scheduled to perform the following safety assessments, as described in Table 2 and Section 7.1.3:

- Physical examination;

- ECG (HR, QRS, PR and QTcF) and vital signs (DBP, SBP and oxygen saturation levels [SpO_2]);
- Recording of AEs, concomitant medications and study restrictions;
- Safety blood assessments (clinical chemistry, haematology, fasting glucose);
- Urine pregnancy test.

If the early termination visit is performed less than 7 days after the intake of the last dose of study treatment, a follow-up call (or visit, if deemed necessary by the Investigator) will also be performed 7 to 10 days after the last dose of study treatment.

In case of discontinuation from the study, the Investigator must fill-in the "Study Termination" page of the eCRF, reporting the main reason for withdrawal.

The subject number assigned to an individual who is withdrawn or who drops out of the study will not be reassigned to another subject.

4.6 SCREEN FAILURES

Screen failures are defined as potential participants who sign the ICF but are not subsequently randomised or entered in the study.

Subjects who do not meet the criteria for participation in the study (screen failures) may be rescreened once: the re-screening is allowed if the impacted criterion-in the Investigator's opinion-linked to a temporary condition. Rescreened participants should be assigned a new participant number for the rescreening event.

5. CONCOMITANT MEDICATIONS

5.1 PERMITTED CONCOMITANT MEDICATIONS

1. Occasional paracetamol (maximum 2 g per day, with a maximum of 10 g per 14 days, for mild non-excluding conditions [see exclusion criterion #2, Section 4.3]);
2. Hormonal contraceptives;
3. Hormonal replacement treatment for post-menopausal women;
4. COVID-19 vaccine during the wash-out periods and at least 7 days before Day 1 of each treatment period.

   Note: Any systemic symptoms (e.g. myalgia, fever, chills, fatigue) after COVID-19 vaccine should subside 2 days before the next treatment period. Otherwise, wash-out period extension should be evaluated on a case-by-case basis.

5.2 NON-PERMITTED CONCOMITANT MEDICATIONS

1. From 3 months before screening and up-to the end of the follow-up period: 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);
2. From 3 weeks before randomisation and up-to the end of the follow-up period: any drug treatment, including prescribed or over-the-counter medicines, vitamins, herbal medicines, homeopathic remedies, etc., with the exception of the allowed concomitant medications specified above.

The wash-out requirements prior to the assessments of MCC and lung function are summarised in Section 4.4.

6. TREATMENTS

The study treatments will be supplied to the investigational centre under the responsibility of the Sponsor, who will also provide the Pharmacist/Investigator with appropriate certificates of analytical conformity.

6.1 TREATMENTS BEING INVESTIGATED

6.1.1 APPEARANCE AND CONTENT

Test treatment: placebo HFA-152a propellant pMDI, presented as:

- Active ingredients: none;
- Excipients: HFA-152a propellant, [redacted];
- Presentation: each canister contains 120 doses;
- Appearance: aluminium canister + grey actuator.

Reference treatment and training kit: placebo HFA-134a propellant pMDI*, presented as:

- Active ingredients: none;
- Excipients: HFA-134a propellant, [redacted];
- Presentation: each canister contains 120 doses;
- Appearance: aluminium canister + grey actuator.

*For this study, CHF 1535 placebo HFA-134a propellant pMDI will be used.

6.1.2 DOSAGE AND ADMINISTRATION

6.1.2.1 Rationale for Dose Selection

The dose of test treatment and reference treatment administered in this study (i.e. 5 inhalations BID for 8 days) corresponds to the maximum total daily dose of HFA-134a propellant allowed for administration with the Clenil pMDI device, according to the current SmPC (for Clenil 100 µg, the maximum allowed daily dose is 1000 µg, administered as 500 µg BID).

6.1.2.2 Dosage

Subjects will receive 5 inhalations BID (morning and evening), for a total of 8 days, of each of the following treatments:

- **Test treatment**: placebo HFA-152a propellant pMDI;
- **Reference treatment**: placebo HFA-134a propellant pMDI.

The administration of the study treatment will occur using two pMDI canisters and two actuators (one for each of the two treatment periods) for each subject.

Investigational Phase

At Day 1 of each treatment period, each subject will receive a treatment box containing 2 pMDI devices: one device with a canister identified with a green sticker on the top, and one device with a

canister identified with a red sticker on the top. Subjects will be advised to use the device with a canister identified with a green sticker. The device with a canister identified with a red sticker should be used only if the one with a green sticker does not function normally.

According to the allocation based on the randomisation list, eligible subjects will be administered multiple doses (5 inhalations BID, for a total of 8 days) of each of the following study treatments in a cross-over design:

- **Test treatment**: placebo HFA-152a propellant via pMDI;
- **Reference treatment**: placebo HFA-134a propellant via pMDI.

The first dose (morning of Day 1) and the last dose (morning of Day 8) of each treatment will be administered at the investigational centre.

From the evening of Day 1 to the evening of Day 7 of each treatment period, subjects will continue the study treatment administration at home. They will use a DRC to record compliance to the study treatment, the use of concomitant medications and the occurrence of AEs (see Section 7.2.7).

6.1.2.3 ADMINISTRATION

The study treatment will be administered by inhalation, using the pMDI device with a canister identified with a green sticker (in case of dysfunction, the pMDI with a canister identified with a red sticker should be used). The instructions to be followed during the administration of the study treatments will be provided in a separate document.

Subjects will be advised not to take the study treatment in the morning of Day 8 of each treatment period.

Treatment administration will be recorded in the eCRF by the Investigator or designee. Any issues occurring during the inhalations as evaluated by the Investigator or designee will be reported in the eCRF.

6.1.2.4 SUBJECT TRAINING

6.1.2.4.1 Training with AIM™ Vitalograph®

At screening, the AIM™ Vitalograph® will be used to train the subjects to use the pMDI inhaler correctly. Inhalation through a specific placebo inhaler (different from the one described in Section 6.1.2.4.2) at the normal range (corresponding to a required peak inhaled flow rate of 30 L/min) will be recorded and evaluated by the AIM™ device, due to its ability to monitor the correct timing of the actuation, proper inhalation time, proper flow and breath holding. Once three consecutive successful inhalations are detected by the AIM™ device, the subjects will be told to continue using the same inspiratory flow rate while inhaling the study treatment. Detailed instructions for use of the AIM™ device are reported inside the commercial package.

Subjects will not be included in the study if the training is not completed successfully. Training evidence will be recorded in the eCRF, including the three AIM™ device results.

6.1.2.4.2 Training with Pressurised Metered Dose Inhaler Placebo

At screening, the correct use of the pMDI device will be explained to the subjects according to the instructions for use (provided in a separate document).

Subjects will be trained by using a placebo HFA-134a inhaler, which is identical from a functional point of view to the device used for the administration of the study treatments (see Section 6.3.2). Therefore, the training will be done with the pMDI device, with the inhalation repeated five times or as many times as necessary until the subject is properly trained.

The training kits will be kept at the investigational centre by the Investigator and will not be dispensed to the subjects. The training kit will be assigned to the subject at screening.

The subject will not be included in the study if the training is not completed successfully. Training evidence will be recorded in the eCRF by the Investigator or designee.

6.2 AUXILIARY TREATMENTS

Not applicable, no auxiliary treatments will be administered during this study.

6.3 PACKAGING

6.3.1 STUDY TREATMENT KIT

Treatment kits will be supplied to the Investigator as subject kits containing two treatment period boxes, each containing two pMDI devices (one with a canister identified with a green sticker, and one with a canister identified with a red sticker).

Treatment kits will be supplied in the following packages:

- **Immediate packaging**: two labelled canisters containing placebo HFA-152a propellant or placebo HFA-134a propellant for inhalation (one canister identified with a green sticker on the top, and one canister identified with a red sticker on the top), plus two labelled actuators;
- **Outer packaging** (period box): labelled box containing two canisters and two actuators to be used at one treatment period;
- **Outer packaging** (subject box): one labelled box containing two period boxes.

6.3.2 TRAINING KIT

Training kits will be supplied to the investigational sites. Each kit will consist of one box containing placebo HFA-134a propellant pMDI. The training kits will be used at screening only (see Table 2) and must be stored as specified in Section 6.8.

Training kits will be supplied in the following packages:

- **Immediate packaging**: one labelled canister containing placebo HFA134a propellant pMDI, plus one labelled actuator;
- **Outer packaging** (subject box): labelled box containing one labelled canister and one labelled actuator.

6.3.3 RESCUE MEDICATION

Not applicable.

6.4 LABELLING

All labelling of immediate and outer packaging for training and treatment kits will be in English and will be compliant with Annex 13 of Volume 4 of the Good Manufacturing Practice [23] and according to the UK local law and regulatory requirements.

6.5 TREATMENT ALLOCATION

A balanced block randomisation list will be generated using a computerised system. Each subject will be assigned to one of the two possible sequences arranged in a 2x2 William design.

Each subject will be identified by a unique 4-digit code (SXXX) assigned at screening.

At randomisation (Day 1 of TP1), each eligible subject will be assigned the lowest randomisation number available (a unique 3-digits code [XXX]), according to a preestablished randomisation list:

- Subjects who do not meet the inclusion criteria and/or who meet the exclusion criteria at screening or prior to randomisation will not be eligible to enter the study. They will be considered as "screen failure subjects";

  Note: Screen failure subjects may be rescreened once. Rescreened subjects should be assigned a new participant number for the rescreening event.
- Subjects who fully meet the inclusion and exclusion criteria at screening or before randomisation will be eligible to enter the study;
- Subjects who prematurely terminate the study will be considered as "drop-out subjects". These subjects cannot be screened again.

Note: If a subject is withdrawn or discontinued from the study after randomisation, the subject code and randomisation number will not be reassigned to another subject.

6.6 TREATMENT CODE

This is an open-label study. The randomisation list will be generated as reported in Section 6.5 and distributed to the contract research organisation (CRO) and the investigational site.

The randomisation list will be kept in the Trial Master File.

6.7 TREATMENT COMPLIANCE

Compliance to each treatment will be evaluated on the basis of the information reported by the Investigator (for the treatment administrations on the morning of Day 1 and the morning of Day 8 of each treatment period) and the subjects' DRC (for the treatment administrations from the evening of Day 1 to the evening of Day 7, see Section 7.2.7).

The evaluation of compliance will be done using the following formula:

$$\frac{\text{TOTAL NUMBER OF ADMINISTERED INHALATIONS}}{\text{TOTAL NUMBER OF SCHEDULED INHALATIONS}} \times 100 = \%\ \text{OF ADMINISTERED INHALATIONS}$$

The total number of scheduled inhalations will be calculated on the basis of the extent of exposure of each subject (8 days for each treatment period). A range of 80% will be taken into account for a satisfactory level of compliance.

6.8 DRUG STORAGE

The Pharmacist/Investigator will be responsible for the safe storage of all medications assigned to this study. Study medications are to be stored in a locked, secure storage facility with access limited to those individuals authorised to dispense them.

The following storage conditions must be respected:

- **Treatment kits** (placebo HFA-152a propellant pMDI and placebo HFA-134a propellant pMDI): the subject box must be stored in a refrigerator at a temperature between 2˚C and 8˚C. In the morning of Day 1 of TP1, only the period box must be removed from the refrigerator, while the subject box must be maintained in the refrigerator until Day 1 of TP2.

  At Day 1 of each treatment period, at discharge, the period box will be dispensed to the subjects. Subjects will be advised to keep the period box at ambient temperature: not above

25°C and not in the refrigerator. At ambient temperature, the residual shelf life of the pMDI kit is of 2 months (60 days). Therefore, once the kit is removed from the refrigerator and before dispensing it to the subject, the Pharmacist/Investigator must write the use-by date (i.e. dispensing date plus 2 months) on the kit labels. The use-by date must not exceed the total shelf life of the product.

- **Training kit** (placebo HFA-134a propellant pMDI): before being used, kits must be stored in a refrigerator, at a temperature between 2°C and 8°C.

  At screening (V1), the training kit to be used must be removed from the refrigerator. At ambient temperature, the residual shelf life of this pMDI is of 3 months (90 days). Therefore, the Pharmacist/Investigator must write the use-by date (i.e. date of the screening visit [V1] plus 3 months) on the kit labels. The use-by date must not exceed the total shelf life of the product.

Once used, the training kits must be kept at the investigational site at ambient temperature (not above 25°C and not in the refrigerator) and should not be dispensed to the subjects.

In case of study medication exposed to temperatures outside the required temperature range, Chiesi or delegate shall issue the assessment of its usability which shall either release the quarantined study drug or declare its permanent non-usability.

6.9 TREATMENT ACCOUNTABILITY AT SITE

The Investigator or a designated/authorised representative is responsible for the management of all the study medications to be used for the study. Study medications should be stored in a locked, secure storage facility with access limited to those individuals authorised 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 medications received, dispensed, and returned by each subject during the study.

At the conclusion or termination of the study, the Investigator or Pharmacist shall conduct and document a final inventory of the drug supply (used and unused). An explanation will be given for any discrepancies.

All the study medications, supplied, used or unused, will be destroyed by the Investigator, directly at the investigational site. In this case, a destruction certificate must be requested from the investigational site and must be filed, both at the investigational site and by the Sponsor. Destruction will not occur until authorised by Chiesi.

6.10 PROVISION OF ADDITIONAL CARE

Not applicable, the study will be conducted in healthy subjects.

6.11 HOME VISITS

Not applicable.

7. STUDY PLAN

7.1 STUDY SCHEDULE

The study will be conducted as described in Section 3 and Table 2.

Current UK national laws and site-specific recommendations for the prevention of the COVID 19 pandemic will be strictly adhered to. These include subjects' monitoring for COVID-19 symptoms

and the adherence to the appropriate measures to control the transmission of the virus in the clinical setting [24].

The study plan and scheduled tests are summarised in Table 2.

The execution order of the study treatment administration and of the cardiac (ECG and vital signs), pulmonary (lung function) and MCC assessments on Day 8 of each treatment period is further specified in Table 3.

Table 2. Schedule of assessments

	Screening visit (V1)	Investigational Phase (TP1 and TP2) [1]				Early termination visit	Follow-up call [2]
		V2/V5	V3/V6	V3 to V4 V6 to V7	V4/V7		
Date	2-21 days before V2	Day -1	Day 1 (morning)	Day 1 (evening) to Day 7 (evening)	Day 8 (morning)		7-10 days after last intake of study treatment
Informed consent	X						
Randomisation			X *(TP1 only)*				
Ambulatory visit	X	X	X		X	X	X[2]
Treatment intake							
Treatment administration [3]			X	X	X		
Training							
Placebo training	X						
Training with AIM™	X						
Subject health evaluation							
Inclusion/Exclusion criteria	X	X *(TP1 only)*	X *(TP1 only)*				
Medical history and previous medications	X						
Demographic data	X						
Height and weight	X						
Body temperature	X	X	X		X	X	
Physical examination	X		X		X (*TP2 only*)	X	
Alcohol breath test	X	X			X	X	
Adverse events recording	X	X	X	X	X	X	X
Restrictions	X	X	X	X	X	X	
Concomitant medications	X	X	X	X	X	X	X

	Screening visit (V1)	Investigational Phase (TP1 and TP2) [1]				Early termination visit	Follow-up call [2]
		V2/V5	V3/V6	V3 to V4 V6 to V7	V4/V7		
Date	2-21 days before V2	Day -1	Day 1 (morning)	Day 1 (evening) to Day 7 (evening)	Day 8 (morning)		7-10 days after last intake of study treatment
Safety assessments in blood							
Clinical chemistry	X				X (*TP2 only*)	X	
Serology	X						
Haematology	X				X (*TP2 only*)	X	
Fasting glucose	X				X (*TP2 only*)	X	
FSH or pregnancy test[4]	X						
Generic assessments in urine							
Urinalysis	X						
Cotinine test	X	X			X	X	
Drug panel test	X	X			X	X	
Pregnancy test [4]		X			X (*TP2 only*)	X	
Cardiac assessments [5]							
12-lead ECG	X		X		X	X	
Vital signs	X		X		X	X	
Pulmonary assessment							
Lung function [6]	X		X		X		
MCC assessment [7]							
Co-57 transmission scan and background scan		X *(TP1 only)*					
Administration of radiotracer and image acquisition		X			X		

	Screening visit (V1)	Investigational Phase (TP1 and TP2) [1]				Early termination visit	Follow-up call [2]
		V2/V5	V3/V6	V3 to V4 V6 to V7	V4/V7		
Date	2-21 days before V2	Day -1	Day 1 (morning)	Day 1 (evening) to Day 7 (evening)	Day 8 (morning)		7-10 days after last intake of study treatment
Other assessments							
Daily record card [8]				X			

BID=Twice daily; Co-57=57-Cobalt; DBP=Diastolic blood pressure; ECG=Electrocardiogram; eCRF=Electronic case report form; FEV_1=Forced expiratory volume in 1 second; FSH=Follicle-stimulating hormone; FVC=Forced vital capacity; MCC=Mucociliary clearance; SBP=Systolic blood pressure; SpO_2=Oxygen saturation levels; TP=Treatment Period; V=Visit; WOCBP=Women of childbearing potential.

[1] TP1 and TP2 will be separated by a 7- to 14-day wash-out period.

[2] A follow-up visit (instead of a call) can be performed, if deemed necessary by the Investigator. A follow-up call (or visit) will be performed in case of early termination as well, if the early termination visit is performed less than 7 days after the intake of the last dose of the study treatment.

[3] A total of 5 inhalations BID for 8 consecutive days, starting from the morning of Day 1 until the morning of Day 8. The first and last dose of the study treatment will be administered at the investigational site.

[4] For logistical reasons, both the pregnancy test (in blood and urine) and FSH test will be performed for samples collected from all female subjects. However, only the relevant results will be recorded in the eCRF (i.e. pregnancy tests for WOCBP and FSH tests for post-menopausal women).

[5] Cardiac assessments will be performed before the lung function assessments and the blood tests, and as described below:

- Vital signs:
  At screening, SBP and DBP (in duplicate, made within a 5-minute interval) will be evaluated after 5 minutes in supine position.
  On the morning of Day 1 and Day 8 of each treatment period, SBP, DBP (in duplicate) and SpO_2 (in single) will be evaluated at the following timepoints:
  - Pre-dose: within 1 hour before the intake of the first inhalation of the propellant;
  - Post-dose: 5 minutes, 15 minutes, 1 hour 45 minutes after the intake of the last inhalation of the propellant.
- Local 12-lead safety ECG: a triplicate ECG will be performed to assess eligibility at screening (V1) and a single ECG will be evaluated on the morning of Day 1 and Day 8 of each treatment period, at the same timepoints (before or after) as the vital signs.

In case of early termination, SpO_2 (single), SBP and DBP (duplicate) will be assessed and a single local 12-lead ECG will be measured during the early termination visit.

	Screening visit (V1)	Investigational Phase (TP1 and TP2) [1]				Early termination visit	Follow-up call [2]
		V2/V5	V3/V6	V3 to V4 V6 to V7	V4/V7		
Date	2-21 days before V2	Day -1	Day 1 (morning)	Day 1 (evening) to Day 7 (evening)	Day 8 (morning)		7-10 days after last intake of study treatment

[6] Lung function assessments (spirometry [FEV_1 and FVC]) will be performed as follow:

- Screening: single spirometry to assess the subject's eligibility;
- On the morning of Day 1 and Day 8 of each treatment period, spirometry assessments will be performed as follow:
  - Pre-dose: within 30 minutes before the intake of the first inhalation of the propellant;
  - Post-dose: 20 minutes after the intake of the last inhalation of the propellant.

[7] MCC assessments will be performed as follow:

- Co-57 transmission scan and background scan: Day -1 of TP 1 only, prior to the assessment of MCC;
- Administration of radiotracer and image acquisition: subjects will be administered a radiotracer and image acquisitions will be performed at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of the radiotracer. The analysis of these images will provide a measurement of MCC.
  On Day 8 of each treatment period, the radiotracer will be administered approximately 2 hours after the administration of the last dose of the propellant.

[8] Subjects will use a daily record card (one for each treatment period) to record the intake of the study propellant, the occurrence of adverse events or the intake of concomitant medications. The daily record card will be given to the subjects on Day 1 of each treatment period and will be collected on Day 8 of each treatment period. In case of early discontinuation, if discontinuation occurs during a treatment period, the daily record card will be collected during the early termination visit.

Table 3. Assessment execution order on Day 8 of each treatment period

Execution order	Task	Timepoint (± acceptable time window)	Reference time
1	**Pre-dose ECG and vital signs**	Within 1 h	First inhalation of propellant
2	**Pre-dose lung function**	Within 30 min	
3	**Inhalation of propellant**	NA	
4	**ECG and vital signs**	5 min ± 5min	Last inhalation of propellant
5	**ECG and vital signs**	15 min ± 5 min	
6	**Lung function**	20 min ± 5 min	
7	**ECG and vital signs**	1h 45 min ± 15 min	
8	**Administration of radiotracer**	Approximately 2 h	
9	**Image acquisition**	0 min ± 2 min	End of radiotracer inhalation
10	**Image acquisition**	15 min ± 5 min	
11	**Image acquisition**	30 min ± 5 min	
12	**Image acquisition**	1 h ± 5 min	
13	**Image acquisition**	1 h 30 min ± 5 min	
14	**Image acquisition**	2 h ± 5 min	
15	**Image acquisition**	2 h 30 min ± 5 min	
16	**Image acquisition**	3 h ± 5 min	
17	**Image acquisition**	4 h ± 5 min	

ECG=Electrocardiogram; h=hour; min=minute; NA=Not applicable.

7.1.1 SCREENING (V1)

A screening visit will be carried-out in order to identify eligible consenting subjects for the study.

The screening visit will take place 2 to 21 days before the start of the Investigational Phase (Day -1 of TP1).

The following procedures will take place at screening:

- Informed consent: a signed ICF will be obtained from potential eligible subjects, after a full and detailed description of the study by the Investigator. The Investigator or his/her designee should provide subjects with ample time and opportunity to inquire about details of the study and to decide whether or not to participate in the study. The ICF has to be signed before the start of any study-related procedures (see Section 15);
- Demographic data collection: demographic data including age, gender and race will be recorded;
- Physical examination: a full physical examination will be performed;
- Medical/surgical history, concomitant diseases and previous medications: subjects' medical and surgical history, as well as concomitant diseases (i.e. diseases pre-existing at the time of the ICF signature) will be recorded. Any medications taken in the 3 months preceding the screening visit will be recorded;
- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the spirometry assessments and blood tests will be checked according to Section 4.4;
- Concomitant medications: any medication taken regularly at the time of this visit will be recorded;
- AE recording: AEs that have occurred since the ICF signature will be recorded in the eCRF, along with the related concomitant medications.
  In cases where clinically significant (CS) abnormalities are revealed during the physical examination or screening procedures, these will be recorded in the subject's eCRF as medical history, unless the start date/time is after the ICF signature date/time and it is not due to a pre-existing condition. In the latter case, it will be recorded in the eCRF as an AE;
- Local safety ECG: triplicate 12-lead ECG will be used to measure HR, QRS, PR and QTcF. The assessment will be performed according to Section 7.2.4;
- Vital signs: SBP and DBP will be measured according to Section 7.2.3;
- Body temperature: will be assessed according to Section 7.2.3;
- Blood test: blood samples will be collected for safety evaluations (clinical chemistry, serology, haematology and fasting glucose), after the 12-lead ECG and vital signs assessments, according to Section 7.2.5;
- FSH or pregnancy tests: blood samples will be collected from female subjects for FSH and serum pregnancy tests, according to Section 7.2.5;
- Urine test: urine samples will be collected for urinalysis and for urine cotinine and drug panel test (according to Section 7.2.6);
- Alcohol breath test: will be performed using a specific kit;
- Height and weight: will be recorded;
- Lung function: single spirometry will be performed (after the ECG records and the assessment of the vital signs), according to Section 7.2.2;

- Training: subjects will receive training on the proper use of the inhaler with the AIM™ Vitalograph and a pMDI placebo, according to Section 6.1.2.4.1 and Section 6.1.2.4.2, respectively;
- Subject selection: inclusion/exclusion criteria will be checked (see Section 4.2 and Section 4.3, respectively).

Before discharge, subjects will be given the following instructions:

- To adhere to the study restrictions and to the pre-MCC wash-out requirements before coming to V2, according to Section 4.4;
- To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2 INVESTIGATIONAL PHASE

The Investigational Phase will comprise two 9-day treatment periods (TP1 and TP2), separated by 7 to 14 days of wash-out from the propellant. Each treatment period will consist of 3 ambulatory visits at the investigational site.

7.1.2.1 Treatment Period 1

7.1.2.1.1 Day -1 of Treatment Period 1 (V2)

V2 is the first visit of the Investigational Phase and of TP1. It takes place 2 to 21 days after the screening visit.

The following procedures will take place at this ambulatory visit:

- Subject selection: some of the inclusion and exclusion criteria will be re-checked (see Section 4.2 and Section 4.3, respectively). Subjects not fulfilling the inclusion criteria or meeting any of the exclusion criteria will be recorded as screening failures;
- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the MCC assessment will be checked, according to Section 4.4;
- Alcohol breath test: will be performed using a specific kit;
- Concomitant medications: concomitant medications taken by the subject since the screening visit and during V2 will be checked and recorded;
- Urine test: urine samples will be collected for urine cotinine and drug panel test, according to Section 7.2.6;
- Pregnancy test: urine samples will be collected from female subjects for pregnancy testing, according to Section 7.2.6;
- Body temperature: will be assessed according to Section 7.2.3;
- AE recording: the status of AEs ongoing at the screening visit will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during the study visit must also be monitored and recorded in the eCRF;
- MCC assessment: a Co-57 transmission scan and a background scan will be performed to provide an outline of the lungs. Subjects will then be administered a radiotracer. Image acquisitions for the assessment of MCC will be performed at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of the radiotracer, according to Section 7.2.1.

Before discharge, subjects will be given the following instructions:

- To adhere to the study restrictions and to the pre-spirometry wash-out requirements before coming to V3, according to Section 4.4;
- To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2.1.2 Day 1 of Treatment Period 1 (V3)

V3 is the second visit of TP1 and takes place in the morning of the day after V2.

The following schedule will be followed during this ambulatory visit:

- Subjects will undergo the necessary assessments and checks to confirm their eligibility;
- Eligible subjects will be randomised;
- Randomised subjects will undergo the pre-dose assessments;
- Subjects will receive the first dose of one of the study treatments (HFA-152a or HFA-134a, according to their randomisation arm);
- Subjects will undergo the post-dose assessments;
- Subjects will be given the necessary instructions before discharge.

The following procedures and assessments will take place:

- Subject selection: specific inclusion and exclusion criteria will be re-checked (see Section 4.2 and Section 4.3, respectively). Subjects not fulfilling the inclusion criteria or meeting any of the exclusion criteria will be recorded as screening failures;
- Randomisation: subjects will be randomised according to Section 6.5;
- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the spirometry assessment will be checked, according to Section 4.4;
- Concomitant medications: concomitant medications taken by the subject since the previous visit and during V3 will be checked and recorded;
- AE recording: the status of AEs ongoing at V2 will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during this study visit must also be monitored and recorded in the eCRF;
- Physical examination: a full physical examination will be performed, and any new CS abnormality revealed since the screening visit (V1) will be recorded as an AE;
- 12-lead ECG: a single ECG will be recorded to assess ECG parameters (HR, PR, QRS and QTcF), according to Section 7.2.4. The assessment will be performed before or after the vital signs assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes, 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Vital signs: SBP, DBP and SpO_2 will be measured according to Section 7.2.3. The assessments will be performed before or after the 12-lead ECG assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Body temperature: will be assessed according to Section 7.2.3;
- Lung function: spirometry assessments will be performed according to Section 7.2.2. Spirometry assessments will be performed in pre-dose: within 30 minutes before the intake of

the first inhalation of the propellant; and in post-dose: 20 minutes after the intake of the last inhalation of the propellant;

- Study treatment administration: single-dose (5 inhalations) administration of one of the study propellants (based on each subject's randomisation arm), according to Section 6.1.2.3;
- DRC: subjects will be given the DRC of TP1. The DRC will be used for the recording of compliance to the study treatment, the use of concomitant medications, and the occurrence of AEs, according to Section 7.2.7.

Before discharge, subjects will receive the following instructions:

- Study treatment administration: subjects will be instructed to continue taking the study treatment (5 inhalations, BID [morning and evening]) from the evening of Day 1 up-to the evening of Day 7, according to Section 6.1.2.3;
- DRC: subjects will be instructed to record each intake of the study treatment, as well as the use of concomitant medications and occurrence of AEs, according to Section 7.2.7. Subjects will also be reminded to bring back the DRC at the next visit;
- Restrictions: subjects will be instructed:
  - Not to take the morning dose of Day 8 before coming to V4;
  - To adhere to the study restrictions and to the pre-spirometry and pre-MCC wash-out requirements before coming to V4, according to Section 4.4;
  - To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2.1.3 Day 8 of Treatment Period 1 (V4)

V4 is the third (last) visit of TP1 and starts in the morning of Day 8.

The following schedule will be followed during this ambulatory visit:

- DRCs will be collected;
- Subjects will undergo the pre-dose assessments;
- Subjects will receive the last dose of the study treatment received during TP1 (HFA-152a or HFA-134a, based on the randomisation arm);
- Subjects will undergo the post-dose assessments, including the administration of a radiotracer and the performance of the MCC assessment;
- Subjects will be given the necessary instructions before discharge.

The following procedures and assessments will take place:

- DRC: the Investigator (or designee) will collect the DRC of TP1;
- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the spirometry and MCC assessments will be checked, according to Section 4.4;
- Alcohol breath test: will be performed using a specific kit;
- Concomitant medications: concomitant medications taken by the subject since the previous visit and during V4 will be checked and recorded;
- Urine test: urine samples will be collected for urine cotinine and drug panel test, according to Section 7.2.6;
- AE recording: the status of AEs ongoing at V3 will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in

the eCRF, as well as the related concomitant medications. Any AE occurring during this study visit must also be monitored and recorded in the eCRF;

- 12-lead ECG: a single ECG will be recorded to assess ECG parameters (HR, PR, QRS and QTcF), according to Section 7.2.4. The assessment will be performed before or after the vital signs assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Vital signs: SBP, DBP and SpO_2 will be measured according to Section 7.2.3. The assessment will be performed before or after the 12-lead ECG assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Body temperature: will be assessed according to Section 7.2.3;
- Lung function: spirometry assessments will be performed according to Section 7.2.2. Spirometry assessments will be performed in pre-dose: within 30 minutes before the intake of the first inhalation of the propellant; and in post-dose: 20 minutes after the intake of the last inhalation of the propellant;
- Study treatment administration: single-dose (5 inhalations) treatment administration, according to Section 6.1.2.3, of the study treatment administered at TP1;
- MCC assessment: subjects will be administered a radiotracer approximately 2 hours after the administration of the last dose of the propellant, and image acquisitions for the assessment of MCC will be performed at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of a radiotracer, according to Section 7.2.1.

The execution order of the study treatment administration and of the cardiac (ECG and vital signs), pulmonary (lung function) and MCC assessments on Day 8 of each treatment period is further specified in Table 3. Subjects will be served a standardised small lunch approximately 2 hours after the administration of the radiotracer.

TP1 is followed by a 7- to 14-day wash-out period from the propellant.

Before discharge, subjects will be given the following instructions:

- To adhere to the study restrictions and to the pre-MCC wash-out requirements before coming to V5, according to Section 4.4;
- To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2.2 Treatment Period 2

7.1.2.2.1 Day -1 of Treatment Period 2 (V5)

V5 is the first visit of TP2. It takes place after a 7- to 14-day wash-out period from the propellant administered during TP1.

The following procedures will take place at this ambulatory visit:

- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the MCC assessment will be checked, according to Section 4.4;
- Alcohol breath test: will be performed using a specific kit;
- Concomitant medications: concomitant medications taken by the subject since the previous visit and during V5 will be checked and recorded;

- Urine test: urine samples will be collected for urine cotinine and drug panel test, according to Section 7.2.6;
- Pregnancy test: urine samples will be collected from female subjects for pregnancy testing, according to Section 7.2.6;
- Body temperature: will be assessed according to Section 7.2.3;
- AE recording: the status of AEs ongoing at V4 will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during the study visit must also be monitored and recorded in the eCRF;
- MCC assessment: subjects will be administered a radiotracer. Image acquisitions for the assessment of MCC will be performed at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of a radiotracer, according to Section 7.2.1.

Before discharge, subjects will be given the following instructions:

- To adhere to the study restrictions and to the pre-spirometry wash-out requirements before coming to V6, according to Section 4.4;
- To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2.2.2 Day 1 of Treatment Period 2 (V6)

V6 is the second visit of TP2 and will take place in the morning of the day after V5.

The following schedule will be followed during this ambulatory visit:

- Subjects will undergo the pre-dose assessments;
- Subjects will receive the first dose of the other study treatment (HFA-152a or HFA-134a, following a cross-over design [Figure 1]);
- Subjects will undergo the post-dose assessments;
- Subjects will be given the necessary instructions before discharge.

The following procedures and assessments will take place:

- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the spirometry assessment will be checked, according to Section 4.4;
- Concomitant medications: concomitant medications taken by the subject since the previous visit and during V6 will be checked and recorded;
- AE recording: the status of AEs ongoing at V5 will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during this study visit must also be monitored and recorded in the eCRF;
- Physical examination: a full physical examination will be performed, and any new CS abnormality revealed since the previous physical examination (performed at V3) will be recorded as an AE;
- 12-lead ECG: a single ECG will be recorded to assess ECG parameters (HR, PR, QRS and QTcF), according to Section 7.2.4. The assessment will be performed before or after the vital signs assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;

- Vital signs: SBP, DBP and SpO_2 will be measured according to Section 7.2.3. The assessment will be performed before or after the 12-lead ECG assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Body temperature: will be assessed according to Section 7.2.3;
- Lung function: spirometry assessments will be performed according to Section 7.2.2. Spirometry assessments will be performed in pre-dose: within 30 minutes before the intake of the first inhalation of the propellant; and in post-dose: 20 minutes after the intake of the last inhalation of the propellant;
- Study treatment administration: single-dose (5 inhalations) administration of the other propellant (following a cross-over design), according to Section 6.1.2.3;
- DRC: subjects will be given the DRC of TP2. The DRC will be used for the recording of compliance to the study treatment, the use of concomitant medications, and the occurrence of AEs, according to Section 7.2.7.

Before discharge, subjects will receive the following instructions:

- Study treatment administration: subjects will be instructed to continue taking the study treatment (5 inhalations, BID [morning and evening]) from the evening of Day 1 up-to the evening of Day 7, according to Section 6.1.2.3;
- DRC: subjects will be instructed to record each intake of the study treatment, as well as the use of concomitant medications and occurrence of AEs, according to Section 7.2.7. Subjects will also be reminded to bring back the DRC at the next visit;
- Restrictions: subjects will be instructed:
  - Not to take the morning dose of Day 8 before coming to V7;
  - To adhere to the study restrictions and to the pre-spirometry, pre-MCC and pre-blood test wash-out requirements before coming to V7, according to Section 4.4;
  - To avoid taking any of the non-permitted concomitant medications, according to Section 5.2.

7.1.2.2.3 Day 8 of Treatment Period 2 (V7)

V7 is the third (last) visit of TP2 and of the Investigational Phase, and will take place in the morning of Day 8.

The following schedule will be followed during this ambulatory visit:

- DRCs will be collected;
- Subjects will undergo the pre-dose assessments;
- Subjects will receive the last dose of the study treatment administered during TP2 (HFA-152a or HFA-134a);
- Subjects will undergo the post-dose assessments, including the administration of a radiotracer and the performance of the MCC assessment.

The following procedures and assessments will take place:

- DRC: the Investigator (or designee) will collect the DRC of TP2;
- Study restrictions: study restriction criteria and adherence to the wash-out requirements applicable before the spirometry and MCC assessments and blood tests will be checked, according to Section 4.4;
- Alcohol breath test: will be performed using a specific kit;

- Concomitant medications: concomitant medications taken by the subject since the previous visit and during V7 will be checked and recorded;
- Urine test: urine samples will be collected for urine cotinine and drug panel test, according to Section 7.2.6;
- Pregnancy test: urine samples will be collected from female subjects for pregnancy testing, according to Section 7.2.6;
- Body temperature: will be assessed according to Section 7.2.3;
- AE recording: the status of AEs ongoing at V6 will be checked and updated in the eCRF when applicable. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during this study visit must also be monitored and recorded in the eCRF;
- Physical examination: a full physical examination will be performed, and any new CS abnormality revealed since the previous visit will be recorded as an AE;
- 12-lead ECG: a single ECG will be recorded to assess ECG parameters (HR, PR, QRS and QTcF), according to Section 7.2.4. The assessment will be performed before or after the vital signs assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Vital signs: SBP, DBP and SpO_2 will be measured according to Section 7.2.3. The assessment will be performed before or after the 12-lead ECG assessment, in pre-dose: within 1 hour before the intake of the first inhalation of the propellant; and in post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant;
- Blood test: blood samples will be collected for safety evaluations (clinical chemistry, haematology and fasting glucose), after the 12-lead ECG and vital signs assessments, according to Section 7.2.5;
- Lung function: spirometry assessments will be performed according to Section 7.2.2. Spirometry assessments will be performed in pre-dose: within 30 minutes before the intake of the first inhalation of the propellant; and in post-dose: 20 minutes after the intake of the last inhalation of the propellant;
- Study treatment administration: single-dose (5 inhalations) treatment administration, according to Section 6.1.2.3, of the study propellant administered at TP2;
- MCC assessment: subjects will be administered a radiotracer approximately 2 hours after the administration of the last dose of the propellant, and image acquisitions for the assessment of MCC will be performed at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of a radiotracer, according to Section 7.2.1.

The execution order of the study treatment administration and of the cardiac (ECG and vital signs), pulmonary (lung function) and MCC assessments on Day 8 of each treatment period is further specified in Table 3. Subjects will be served a standardised small lunch approximately 2 hours after the administration of the radiotracer.

A follow-up call (or visit, if deemed necessary by the Investigator) will be performed 7 to 10 days after V7, according to Section 7.1.4.

7.1.3 EARLY TERMINATION VISIT

In the case of early discontinuation (except when the reason for early discontinuation is lost to follow-up or consent withdrawal, and except when the subject is unwilling to perform an early termination visit), an early termination visit will be performed in order to assess the subject's safety.

The following assessments will be performed during this ambulatory visit:

- DRC: in case early termination occurs during a treatment period, the Investigator (or designee) will collect the subject's DRC;
- Alcohol breath test: will be performed using a specific kit;
- Concomitant medications: concomitant medications taken by the subject since the last study visit and during the early termination visit will be checked and recorded;
- Urine test: urine samples will be collected for urine cotinine and drug panel test, according to Section 7.2.6;
- AE recording: the status of any AEs ongoing at the previous visit will be checked and updated in the eCRF, along with the related concomitant medications. Any new AE occurring since the previous visit will be checked and recorded in the eCRF, as well as the related concomitant medications. Any AE occurring during the study visit must also be monitored and recorded in the eCRF;
- Pregnancy test: urine samples will be collected from female subjects for pregnancy testing, according to Section 7.2.6;
- Physical examination: a full physical examination will be performed and any new CS abnormality revealed since the previous physical examination will be recorded as an AE;
- 12-lead ECG: a single ECG will be recorded to assess ECG parameters (HR, PR, QRS and QTcF), according to Section 7.2.4;
- Vital signs: SBP, DBP and SpO_2 will be measured after 5 minutes in supine position, as described in Section 7.2.3;
- Body temperature: will be assessed according to Section 7.2.3;
- Blood test: blood samples will be collected for safety evaluations (clinical chemistry, haematology and fasting glucose), after the 12-lead ECG and vital signs assessments, according to Section 7.2.5. Subjects will have to fast for at least 10 hours before blood samples are collected.

If the early termination visit is performed less than 7 days after the intake of the last dose of study treatment, a follow-up call (or visit, if deemed necessary by the Investigator) will also be performed 7 to 10 days after the last dose of study treatment.

7.1.4 FOLLOW-UP

A follow-up call (or visit, if deemed necessary by the Investigator) will be performed 7 to 10 days after the last intake of the study propellant for a safety follow-up.

The following assessments will be performed during this call/visit:

- AE recording: the status of any AEs ongoing at V7 (or the early termination visit) will be checked and updated in the eCRF, along with the related concomitant medications. Any new AE occurring since the last visit will be checked and recorded in the eCRF, as well as the related concomitant medications;

- Concomitant medications: concomitant medications taken by the subject since V7 (or the early termination visit) will be checked and recorded.

7.2 STUDY ASSESSMENTS AND PROCEDURES

7.2.1 MUCOCILIARY CLEARANCE ASSESSMENT

Image acquisitions for MCC assessments, the transmission scan and the background scan will be performed at the timepoints specified in Table 2.

On Day -1 of TP1 (before the assessment of MCC), the gamma scintigraphy procedure will be performed and will consist of a single transmission scan of the thorax, performed using a planar Co-57 source. The procedure will provide an outline of the lungs for use in subsequent analysis. A single background scan will also be performed during this visit, to account for background radiation. Subjects will be standing in front of the camera and any radioactive substance (except the markers) will be removed from the room.

On each visit with MCC assessments (see Table 2), subjects will be administered 99mTc-albumin colloid via a standardised aerosol delivery procedure. Controlled breathing conditions will be implemented using a metronome and commercial visual flow signal. A Mini 900 Ratemeter will be used to target a dose of 1-2 MBq (maximal dose of 2 MBq per administration). To assure that this adequate dose is achieved, the probe will be shielded with approximately 1 mm lead and will be taken from the middle of a subject's back. Inhalation time will vary by subject size and deposition efficiency, but is expected to be around 2 minutes per subject.

Gamma scintigraphy imaging will be performed over 240 minutes. Image acquisition will begin after completing the 99mTc-albumin administration, and the analysis of these images will provide a measurement of MCC. Anterior and posterior images of a 2-minute duration will be taken at 0 (immediately), 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after the administration of the radiotracer. In order to allow accurate alignment of sequential images, an external anatomical marker containing a maximum of 0.02 MBq 99mTc will be taped to the subjects' chest and back, for the duration of the imaging procedures.

On Day 8 of each treatment period, the radiotracer will be administered post-dose, approximately 2 hours after administration of the last dose of the propellant.

Images will be reviewed and counts in each region of interest will be captured and corrected for background and radiation decay. To assess central *versus* peripheral deposition, the lung will be divided into central and peripheral (the area outside central) regions.

The procedure for the image acquisition and the measurement of MCC will be described in the laboratory manual.

The following parameters will be collected from the MCC measurements at each timepoint specified in Table 2:

- PPR in whole lung;
- PPR in central lung region;
- PPR in peripheral lung region;
- Central/peripheral ratio.

The final data reporting will include the baseline measurements and measurements taken following each treatment period.

The acquisition of the emission scans should start within the following time deviations from the end of the radiotracer inhalation:

- 0 (immediately) after: + 2 minutes;
- 15 minutes, 30 minutes, 1 hour, 1 hour 30 minutes, 2 hours, 2 hours 30 minutes, 3 hours and 4 hours after: ± 5 minutes.

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

7.2.2 LUNG FUNCTION ASSESSMENT

The lung function measurements will be assessed locally at the timepoints specified in Table 2.

Lung function measurements will be performed according to the recommendations of the Official Statement of the European Respiratory Society and American Thoracic Society [25]. Subjects should be resting for at least 10 minutes before the lung function measurements and, during the measurement, they should be in sitting position with their nose clipped. Values will be corrected for body temperature, ambient pressure, gas saturated with water vapor conditions. Calibration of the spirometer must be performed by the same Investigator or designee (to the extent possible) and the reports must be kept with the study source documents.

The following parameters will be recorded:

- FEV_1 (L);
- FVC (L);
- Predicted FEV_1;
- FEV_1 percentage of predicted normal value;
- FEV_1/FVC ratio.

Predicted values of FEV_1 will be calculated according to formulas reported by Quanjer et al. [26].

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

In case abnormal lung function measurements were obtained at screening, (see inclusion criterion #9 in Section 4.2 for details), the subject will be asked to return to the clinic once before randomisation to repeat the test. If, at that time, FEV_1/FVC > 0.70 and $FEV_1 \geq 80\%$ predicted, the subject can be included in the study. Otherwise, the subject will be discharged and recorded as a screening failure.

The spirometer used at the site will provide FEV_1/FVC values as a ratio. For eligibility purposes, values will be considered as acceptable if FEV_1/FVC is > 0.70.

The restrictions to be applied before spirometry assessments are described in Section 4.4.

Assessments on Day 1 and Day 8 of TP1 and TP2 should be performed at the following timepoints:

- Pre-dose: within 30 minutes before the intake of the first inhalation of the propellant;
- Post-dose: 20 minutes after the intake of the last inhalation of the propellant.

Acceptable time deviations from theoretical post-dose timepoints are (actual time to be recorded is the time when the first lung function manoeuvre starts):

- 20 minutes post-dose (last inhalation): ± 5 minutes.

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

7.2.3 VITAL SIGNS

Vital signs (SBP, DBP and SpO_2) and body temperature will be assessed at the timepoints specified in Table 2.

Before the vital signs assessments, subjects should be resting in a quiet, supervised setting with minimal stimulation (e.g. no television, loud music, computer games) and, whenever possible, should lay in a supine position for 5 minutes before each assessment. Vital signs will be assessed either before or after the ECG recordings, and must be assessed before the lung function assessments and the blood tests, as specified in Table 2.

At screening, SBP and DBP will be measured in duplicate (made within a 5-minute interval), and the average of the duplicate values will be used to assess subject eligibility, according to inclusion criterion #7 (see Section 4.2). In case abnormal blood pressure results are obtained at screening, the subject will be asked to return to the clinic once before randomisation, to repeat the blood pressure assessment. If, at that time, the averages of the duplicate parameters are within the allowed ranges, the subject can be included in the study. Otherwise, the subject will be discharged and recorded as a screening failure.

The vital signs assessments planned during the Investigational Phase (Day 1 and Day 8 of TP1 and TP2) and the early termination visit (in case of early termination) are duplicate SBP, DBP and single SpO_2.

Assessments on Day 1 and Day 8 of TP1 and TP2 should be performed at the following time points:

- Pre-dose: within 1 hour before the intake of the first inhalation of the propellant;
- Post-dose: 5 minutes, 15 minutes, and 1 hour 45 minutes after the intake of the last inhalation of the propellant.

Acceptable time deviations from theoretical post-dose timepoints are:

- 5 minutes and 15 minutes post-dose (last inhalation): ± 5 minutes;
- 1 hour 45 minutes post-dose (last inhalation): ± 15 minutes.

Tympanic body temperature will be measured at the timepoints specified in Table 2. Results obtained at screening will be used to check the subjects' eligibility (see Section 4.2, inclusion criterion #7).

Vital signs (SpO_2, SBP and DBP [single values and the average of duplicate measurements, as applicable]) and body temperature values will be reported in the eCRF.

7.2.4 ELECTROCARDIOGRAM ASSESSMENTS

ECG assessments will be performed at the timepoints specified in Table 2.

Subjects should be resting in a quiet, supervised setting with minimal stimulation (e.g. no television, loud music, computer games) and, whenever possible, should lay in a supine position for 5 minutes before each assessment. ECG recordings will be performed either before or after the assessment of vital signs, and must be performed before the lung function assessments and the blood tests, as specified in Table 2.

At screening, a triplicate 12-lead digitised ECG recording will be performed. The average of the triplicate recordings will be used to assess the subjects' eligibility (see exclusion criterion #8 in Section 4.2). In case abnormal ECG results are obtained at screening, the subject will be asked to return to the investigational site once before randomisation to repeat the ECG measurement. If, at that time, the averages of the triplicate ECG parameters are within the allowed ranges, the subject can be included in the study. Otherwise, the subject will be discharged and recorded as a screening failure.

The ECG assessments planned during the Investigational Phase (Day 1 and Day 8 of TP1 and TP2) and the early termination visit will consist in single ECGs, and will be performed for safety assessments.

ECG assessments on Day 1 and Day 8 of TP1 and TP2 should be performed at the following time points:

- Pre-dose: within 1 hour before the intake of the first inhalation of the propellant;
- Post-dose: 5 minutes, 15 minutes and 1 hour 45 minutes after the intake of the last inhalation of the propellant.

Acceptable time deviations from theoretical post-dose timepoints are:

- 5 minutes and 15 minutes post-dose (last inhalation): ± 5 minutes;
- 1 hour 45 minutes post-dose (last inhalation): ± 15 minutes.

The values of all ECG parameters and the overall ECG evaluation will be reported in the eCRF.

7.2.5 BLOOD SAMPLE COLLECTION FOR SAFETY EVALUATION

Subjects should be fasting for at least 10 hours before the blood samples are collected (see Section 4.4). Moreover, blood sample collection should be done after the ECG and blood pressure assessments (before the subject is discharged from the clinic).

At screening, blood sample collections and blood safety evaluations will be performed as follow:

- 5 mL of blood will be collected for clinical chemistry testing (creatinine, urea, aspartate aminotransferase [AST], alanine aminotransferase [ALT], gamma-glutamyl transpeptidase [γ-GT], total bilirubin, alkaline phosphatase, sodium, potassium, albumin);
- 4 mL of blood will be collected for haematology testing (red blood cell [RBC] count, white blood cell [WBC] count and differential, total haemoglobin [Hb], haematocrit [Hct], platelets [PLT] count);
- 5 mL of blood will be collected for:
  - Serology testing (HIV1, HIV2, hepatitis B and hepatitis C);
  - Serum pregnancy test and FSH test.

    Note: For logistical reasons, both the pregnancy test and FSH test will be performed by the local laboratory for blood samples collected from all female subjects. However, only the relevant results will be recorded in the eCRF (i. e. pregnancy tests for WOCBP and FSH tests for post-menopausal women).
- 2 mL of blood for will be collected for the assessment of fasting glucose.

In case abnormal laboratory values are obtained at screening that could indicate a temporary condition (see exclusion criterion #3 in Section 4.3 for details), the subject will be asked to return to the clinic once before randomisation, to repeat the test. If, at that time, no abnormalities are observed, the subject can be included in the study, otherwise the subject will be discharged and recorded as a screening failure.

On Day 8 of TP2, or during the early termination visit (in case of early termination), blood samples will be collected and blood safety evaluations will be performed as follow:

- 5 mL of blood for clinical chemistry testing (creatinine, urea, AST, ALT, γ-GT, total bilirubin, alkaline phosphatase, albumin, sodium, potassium);
- 4 mL of blood for haematology testing (RBC count, WBC count and differential, total Hb, Hct, PLT count);
- 2 mL of blood for fasting glucose.

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

7.2.6 URINE SAMPLE COLLECTION FOR SAFETY EVALUATION

A single urine sample of at least 20 mL will be collected at screening, and the following analyses will be performed:

- Dipstick urinalysis: bilirubin, urobilinogen, ketones, glucose, proteins, blood, nitrite, potential of hydrogen (pH), specific gravity, and leukocytes;
- Point of care drugs of abuse: methamphetamine, cocaine, cannabis, amphetamines, morphine/opiates, benzodiazepines, methylenedioxymethamphetamine, methadone, tramadol and ketamine;
- Point of care urine sample for cotinine.

A single urine sample of at least 20 mL will be collected on Day -1 and Day 8 of each treatment period (or the early termination visit, in case of early termination), and the following analyses will be performed:

- Point of care pregnancy test (except for Day 8 TP1);

  Note: For logistical reasons, the urine pregnancy test will be performed for urine samples collected from all female subjects. However, only the relevant results (pregnancy tests for WOCBP) will be recorded in the eCRF.
- Point of care drugs of abuse: methamphetamine, cocaine, cannabis, amphetamines, morphine/opiates, benzodiazepines, methylenedioxymethamphetamine, methadone, tramadol and ketamine;
- Point of care urine sample for cotinine.

  Note: In case of positive results for cotinine and drug tests at screening or prior to randomisation, the tests can be repeated once at Investigator's discretion.

Urine samples will be collected and prepared according to the instructions provided in the test kits. Results of these assessments will be entered in the eCRF by the Investigator or designee.

7.2.7 DAILY RECORD CARD

Subjects will be given a DRC at Day 1 of each treatment period and will be instructed on how to complete it. Subjects will also be asked to bring the completed DRC with them to the next visit at the investigational centre (i.e. Day 8 of that treatment period). In case a subject discontinues from the study during a treatment period, he/she will be asked to bring the DRC at the early termination visit.

As indicated in Table 2, subjects will use the DRC (one for each treatment period) to record the intake of the study propellant (for the assessment of treatment compliance). Subjects will also record the use of any concomitant medication and the occurrence of any AE (for the assessment of treatment safety).

Data collected throughout the DRC will be entered in the eCRF, as described in Section 11.

8. STUDY ASSESSMENTS

The **primary objective** of this study is to assess the effect of multiple doses of the HFA-152a propellant and the HFA-134a propellant (when administered not in combination with active compounds) on MCC.

The MCC assessment will be performed on Day -1 and on Day 8 of each treatment period, as described in Section 7.2.1.

The following **study variables** will be considered to **assess the primary objective** of the study:

- MCC rate, as assessed by the percent particle retention at 2 hours after the inhalation of radiolabelled particles (PPR_2);
- MCC rate, as assessed by the percent particle retention at 4 hours after the inhalation of radiolabelled particles (PPR_4).

Note: PPR in whole lung will be used for the assessment of the variables above.

The following **study variable** will **complete the evaluation of the primary objective** of the study:

- MCC, as assessed by the area under the tracheobronchial particle retention curve between 0 and 4 hours (AUC_{0-4}) after the inhalation of radiolabelled particles.

9. SAFETY ASSESSMENTS

The **secondary objective** of the study is to evaluate the general safety and tolerability of the two different propellants.

The general safety and tolerability of the propellants will be assessed during each treatment period, by local safety ECGs (as described in Section 7.2.4), vital signs (as described in Section 7.2.3), lung function assessment (as described in Section 7.2.2) and collection of AEs. Moreover, subjects will use DRCs to report AEs and the use of concomitant medications during the treatment periods (as described in Section 7.2.7).

The following **safety variables** will be considered for the evaluation of the general safety and tolerability of each propellant:

- AEs;
- Vital signs (SBP, DBP and SpO_2);
- 12-lead ECG parameters: HR, QTcF, PR and QRS;
- Lung function parameters: FEV_1 and FVC.

10. ADVERSE EVENT REPORTING

10.1 DEFINITIONS

An **Adverse Event (AE)** is any untoward medical occurrence in a subject to whom a medicinal product is administered and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an 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 (ADR)** is a noxious and unintended response to an IMP related to any dose administered.

Within this study, the causality of an AE has to be considered with regards to the placebo and its excipients.

All AEs judged by either the reporting Investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions.

Adverse reactions could derive from medication errors and uses outside what is foreseen in the protocol in relation to the IMP, including misuse and abuse of the product.

A **Serious Adverse Event (SAE)/Serious ADR** is any untoward medical occurrence or noxious and unintended response to an IMP that, at any dose, falls into one or more of the following categories:

- **Results in death**

  Death is not an AE but an outcome. It is the cause of death that should be regarded as the AE. The only exception to this rule is "sudden death" where no cause has been established; in this instance, "sudden death" should be regarded as the AE 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, 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**

  Hospitalisation 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. Hospitalisation 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 an 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 AE**

  This criterion allows for any situation in which important AEs/adverse 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.

  Medical and scientific judgment should be exercised in deciding whether an event is "serious" in accordance with those criteria.

  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.

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

A **Non-Serious AE/Non-Serious ADR** is an AE or ADR that does not meet the criteria listed above for an SAE/serious ADR.

10.2 EXPECTEDNESS

Since no active ingredient will be included in the study treatment but only pMDI containing placebo propellant and excipients will be administered, there is no reference document for the assessment of the expectedness of AEs in this study. All AEs assessed as related to the study treatments and/or their excipients will be considered as unexpected AEs for safety reporting purposes.

10.3 INTENSITY OF ADVERSE EVENT

Each AE 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

In determining whether an AE is an ADR, consideration shall be given to whether there is a reasonable possibility of establishing a causal relationship between the event and the IMP (to be intended as both the tested product and the reference product), based on an analysis of available evidence.

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

Is there a reasonable possibility of relatedness to study drug?

- *Yes*
- *No*

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 (prior or concomitant);

- Withdrawal of study treatment(s);
- Worsening of existing condition;
- Erroneous treatment with study medication (or concomitant medication);
- Protocol-related process.

10.5 ACTION TAKEN WITH THE STUDY DRUG DUE TO AN ADVERSE EVENT

- Dose not changed;
- Treatment permanently withdrawn;
- Treatment temporarily interrupted;
- Unknown;
- Not applicable.

10.6 OTHER ACTIONS TAKEN

- Specific therapy/medication;
- Concomitant procedure.

10.7 OUTCOME

Each AE must be rated by choosing among the following:

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

10.8 RECORDING ADVERSE EVENTS

All AEs occurring during the course of the study must be documented in the Adverse Event page of the eCRF. Moreover, if the AE is serious, the Serious Adverse Event form must be completed as well.

It is the responsibility of the Investigator to collect all AEs (both serious and non-serious) whether collected through subjects' spontaneous, unsolicited reports, by observation, or by routine open questionings.

CS abnormal laboratory findings or other abnormal assessments detected at screening visit not due to a pre-existing condition, or CS changes at the following visits in the medical opinion of the Investigator, must be reported as AEs in the eCRF. If a CS abnormal laboratory finding or another abnormal assessment meets the definition of an AE, then the AE page of the eCRF must be completed, as appropriate.

A diagnosis, if known, or clinical signs and symptoms if diagnosis is unknown, rather than the CS abnormal finding, should be reported on the AE page of the eCRF. If no diagnosis is known and clinical signs and symptoms are not present, then the abnormal finding should be recorded.

The window for recording AEs in the eCRF is the period starting from the informed consent signature until the subject's study participation ends. All AEs must be followed and recorded in the eCRF until this date.

10.9 REPORTING SERIOUS ADVERSE EVENTS TO CHIESI

The Investigator must report all SAEs to the Safety Contact of the CRO [redacted] (later referred to as [redacted]) listed below, without undue delay but not later than within 24 hours of awareness. The information is to be sent by providing the completed SAE 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.

Safety contacts	Telephone no.	Fax no.	E-mail
[redacted] Safety Contact	[redacted]	-	[redacted]
Chiesi Safety Contact	-	+39 05211885003	ct_cds@chiesi.com

- Reporting of SAEs by the Investigator is from the time of the subject's signature of informed consent until the end of the subject's study participation. 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, all SAE reports (both initial and follow-up) are to be reported to the [redacted] Safety Contact. After the site is closed, SAE reports are to be reported directly to the Chiesi Safety Contact.
- For pharmacovigilance purposes, all SAEs must be followed-up until all queries have been resolved, clinical recovery is complete, laboratory results have returned to normal, a stable condition has been reached, or the subject has been lost to follow-up. This is necessary in order to elucidate the nature and/or causality of the SAEs as completely as possible. Follow-up may therefore continue after a subject has left the study. For related SAEs, the follow-up will continue with no timelines, 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.

10.10 REPORTING SERIOUS ADVERSE EVENTS AND OTHER SAFETY ISSUES TO REGULATORY AUTHORITIES/ ETHICS COMMITTEES/ INSTITUTIONAL REVIEW BOARDS

All suspected unexpected serious adverse reactions (SUSARs), which occur with the IMPs within or outside the concerned clinical study, if required, will be reported in compliance with the timelines and standards for reporting SUSARs according to local UK regulations. The Medicines and Healthcare products Regulatory Agency (MHRA) will be informed through MHRA Individual Case Study Report (ICSR) Submission portal, while the EC and the Investigators by Council for International Organizations of Medical Sciences (CIOMS) I form or by periodic line-listings produced by Chiesi Global Pharmacovigilance.

With regard to regulations in force for Pharmacovigilance, the Investigator must fulfil his/her obligation according to the law in force in the UK.

10.11 REPORTING ANNUAL SAFETY REPORTS

Not applicable.

10.12 GENERAL NOTES

- In case of death, a comprehensive narrative report of the case must be prepared by the Investigator and sent to the [redacted] Safety Contact within the Serious Adverse Event form.
- 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 data that could identify a subject must be redacted.
- In case of pregnancy, the subject will be immediately withdrawn from the study and will be asked (with a separate consent) to be followed with due diligence until the outcome of the pregnancy is known and until the child reaches one year of age, in order to detect any congenital anomaly or birth defect. The pregnancy must be reported by the Investigator within 24 hours by fax/e-mail 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 are to be completed by the Investigator with all the available information and sent to the [redacted] Safety Contact. Some of the sections in this form may be completed only if the subject/subject's partner has signed the separate consent form to permit the pregnancy to be followed. 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 the outcome meets the criteria for immediate classification of an SAE (e.g. spontaneous or therapeutic abortion, stillbirth, neonatal death, congenital anomaly, or birth defect), the Investigator must follow the procedure for reporting SAEs.
- If a male subject's partner becomes pregnant during the study, the subject is not to be discontinued from the study/study treatment, but the same reporting procedure is to be followed and the Pregnancy Report Form is to be completed as described above.
- Pregnancies that occur at any time during the study and until the follow-up call (or visit) must be reported.
- If a pregnancy is discovered before a subject has taken the first dose of the study treatment, she must be immediately withdrawn from the study, but the pregnancy does not need to be reported.
- Any ADR occurring with any marketed non-IMP 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 ADRs to the relevant Marketing Authorisation Holders of the involved medicinal products. Additionally, 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.

10.13 DATA MONITORING COMMITTEE

Not applicable.

11. DATA MANAGEMENT

Chiesi electronic data collection system (i.e. eCRF) will be filled-in by the Investigator and/or his/her representative designee based on source documents. Screening failure subjects will not be entered in the eCRF.

Access to electronic systems used for data collection will be granted to the study personnel only after appropriate training.

Subject DRCs will be used during the study and collected on paper; then data will be entered in the eCRF dedicated forms by authorised site personnel.

To ensure data consistency and completeness, data will be cleaned by front-end edit checks running at the time of data collection and by back-end checks used by the Data Manager.

Medical history, AEs, and concomitant procedures will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. Medications will be coded using the World Health Organization (WHO) Drug dictionary and Anatomical Therapeutic Chemical (ATC) classification.

External data (MCC data) will be processed centrally, sent electronically to the designated data management / statistics CRO, and reconciled with the corresponding information recorded in the eCRF.

After data collection, cleaning and coding have been completed, a review meeting will be held to finalise the list of important protocol deviations and to define the analysis sets. 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. Any updates to the study data must be authorised by Chiesi.

At the study conclusion, a complete copy of the study data will be created for archival purposes at Chiesi. The Investigator will receive copies of the subject data for retention at the investigational site.

12. STATISTICAL METHODS

12.1 SAMPLE SIZE

No formal sample size calculation has been performed for this study, considering its exploratory nature. A total of 20 subjects will be randomised in the study in order to reach at least 18 evaluable subjects.

12.2 ANALYSIS SETS

- **Safety set:** all randomised subjects who receive at least one dose of study treatment (analysed as treated).
- **Intention-to-treat set (ITT):** all randomised subjects who receive at least one dose of the study treatment (analysed as randomised).
- **Per-protocol set (PP):** all subjects from the ITT set without any important protocol deviations leading to data exclusion (e.g. wrong inclusions, poor compliance, non-permitted medications).

Exact definition of important protocol deviations impacting the analysis sets will be discussed by the study team during the review of the data and described in the Data Review Report.

Since a cross-over design will be used, the inclusion in the analysis sets will be defined on a per-period basis.

The **study variables** will be analysed in the ITT and PP sets. The **Safety variables** will be analysed in the Safety sets.

12.3 STATISTICAL ANALYSIS

A detailed statistical analysis plan will be described in the Statistical Analysis Plan (SAP). The plan will be finalised before database lock.

12.3.1 IMPORTANT PROTOCOL DEVIATIONS

Important protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of key study data or that may significantly affect a subject's rights, safety, or well-being. Important protocol deviations will be assessed during monitoring activities and derived programmatically from the clinical database. They will include but are not limited to:

- Informed consent issues;
- Critical eligibility criteria not met;
- Intake of non-permitted concomitant medications;
- Subject not enrolled per protocol requirements;
- A significant protocol-required assessment or procedure not performed or wrongly performed;
- Incorrect study treatment intake;
- Study treatment not discontinued despite withdrawal criteria met;
- Safety reporting time frame not respected.

Prior to database lock, the final list of important protocol deviations and their potential impact on the statistical analysis will be documented in the Data Review Report. All important protocol deviations will be summarised using descriptive statistics and presented in the clinical study report (CSR).

12.3.2 SUBJECT DEMOGRAPHICS AND BASELINE CHARACTERISTICS

Demographics and baseline variables will be summarised by sequence and overall, using descriptive statistics for the ITT set. The following variables will be presented: age, gender, race, height, weight, medical history and concomitant diseases, prior and concomitant medications, body temperature, vital signs, 12-lead ECG and lung function. The following variables will be analysed in the PP set as well: demographics (age, gender, race).

Results of the urine and blood laboratory assessments, physical examinations and alcohol breath tests will be listed only.

12.3.3 STUDY VARIABLES

MCC variables (in whole lung) evaluating the primary objective of the study (see Section 8) will be analysed in the ITT and PP sets, as follow:

- The change from baseline in PPR_2 at Day 8 will be analysed using an analysis of covariance (ANCOVA) model, including treatment, subject and period as fixed effects, and PPR_2 at Day -1 as a covariate. The adjusted mean change from baseline obtained with each treatment and the adjusted mean difference between HFA-152a and HFA-134a with their 95% confidence intervals (CIs) and p-values will be estimated by the model;
- The change from baseline in PPR_4 at Day 8 will be analysed using the same model used for the change from baseline in PPR_2.

The MCC variable completing the evaluation of the primary objective will be analysed as follow:

- The change from baseline in AUC_{0-4} after the inhalation of radiolabelled particles will be summarised using descriptive statistics.

Descriptive statistics will also be provided for the MCC data (in whole lung) collected at all other post-dose timepoints (see Section 7.2.1).

Figures will be provided for the mean change from baseline in PPR (in whole lung), by treatment arm and each post-dose timepoints.

Data of other MCC parameters (i.e. MCC on central region and on peripheral region, and central/peripheral ratio, see Section 7.2.1) will be listed only.

12.3.4 SECONDARY STUDY VARIABLES

Not applicable.

12.3.5 SAFETY VARIABLES

Adverse Events

All AEs starting on or after the time of first study drug intake will be classified as treatment-emergent adverse events (TEAEs). Any AEs started after the informed consent signature and before the time of first study drug intake will be classified as pre-treatment AEs.

The number of TEAEs and the number and percentage of subjects who experienced at least one TEAE will be presented for each study treatment for all AEs, ADRs, SAEs, non-serious AEs, serious ADRs, severe AEs, AEs leading to study discontinuation, and AEs leading to death. Summaries will be presented overall and by system organ class (SOC) and preferred term (PT) based on the MedDRA dictionary.

All AEs will be listed. Pre-treatment AEs will be listed only.

ECG

At baseline and at each post-dose timepoint, the following statistics will be presented for each study treatment for the ECG parameters (HR, QRS, PR, QTcF):

- Mean absolute value with its 95% CI;
- Mean change from baseline with its 90% CI (for post-dose time-points only).

For QTcF, the following categories will also be analysed for each study treatment, by means of descriptive statistics:

- For male subjects: QTcF interval > 450 ms, > 480 ms and > 500 ms;
- For female subjects: QTcF interval > 470 ms and > 500 ms;

- For both, male and female subjects: change from baseline > 30 ms and > 60 ms.

The analysis will be presented at each post-dose timepoint and at any post-dose timepoint.

Vital Signs

At baseline and each post-dose timepoint, the following statistics will be presented by treatment for the vital signs parameters (SBP, DBP, SpO_2):

- Mean absolute value with its 95% CI;
- Mean change from baseline with its 95% CI (for post-dose time-points only).

Lung function parameters

Descriptive statistics on actual values and change from baseline will be presented for FEV_1 and FVC. Both absolute and relative changes from baseline will be presented.

12.3.6 EXPLORATORY VARIABLES

Not applicable.

12.3.7 INTERIM ANALYSIS

No interim analysis is planned for this study.

13. ETHICS COMMITTEE/INSTITUTIONAL REVIEW BOARD APPROVAL

The study application will be submitted to the EC in accordance with the UK requirements.

The EC shall give its opinion in writing, clearly identifying the study number, study title, and ICF approved, before the clinical study commences.

A copy of all communications with the EC will be provided to the Sponsor.

The Investigator is to provide written reports to the EC, annually or more frequently if requested by the EC, on any changes that significantly affect the conduct of the study and/or that increase risk to the subjects (according to the UK requirements).

14. REGULATORY AND COMPLIANCE REQUIREMENTS

The study will be notified to the Health Authorities (or authorised by) according to the legal requirements in the UK.

The study will also be authorised by the Administration of Radioactive Substances Advisory Committee (ARSAC), for approval of the dosimetry to be used. The planned procedures are compliant with the Ionising Radiation (Medical Exposure) Regulations, and appropriate review by a Medical Physics Expert and Clinical Radiation Expert have been undertaken.

Selection of the subjects will not start before the approval of the EC has been obtained.

This study will be conducted in compliance with this protocol, the Declaration of Helsinki (1964 and amendments), current ICH E6 GCPs, and all others applicable laws and regulations.

15. INFORMED CONSENT

Informed consent must be written in a language understandable to the subjects. Prior to any study-related procedures taking place, it is the responsibility of the Investigator or a delegate to obtain written consent from each subject by using the latest EC-approved version of the document.

The processing of subjects' data as well as subject inclusion in the study is based on subject explicit consent as pointed out within the informed consent.

Adequate time shall be given to the subject to ask the Investigator or delegate about any clarification needed and to consider his/her decision to participate in the study.

Consent must be documented by the subject with a dated signature. The signature confirms that the individual has understood the information provided. The Investigator or delegate must sign and date the ICF as well. One copy is to be given to the subject and the other is retained by the site.

Subjects who are rescreened will be required to sign a new ICF. A link to the prior subject number will be recorded in the eCRF.

A female subject or the female partner of a male subject who becomes pregnant during the study will be asked to sign a specific ICF to provide permission to Chiesi to collect information about her pregnancy, its outcome, and the health of the child up-to the age of one year. If the partner and/or her legal representative is unable to read, the consent for pregnancy follow-up will be obtained in the presence of an impartial witness who will read the ICF and the written information. The witness shall sign and date the informed consent document.

16. SOURCE DOCUMENTS/DATA

16.1 RECORDING OF SOURCE DATA

Data collection is the responsibility of the clinical study 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 must be completed in a neat, legible manner to ensure accurate interpretation of data.

Data entered in the eCRF-derived from source documents must be consistent with the data recorded on the source documents.

16.2 DIRECT ACCESS TO SOURCE DOCUMENT/DATA

The Investigator or a designee must permit study-related monitoring, audits, EC review or regulatory inspection, providing direct access to source data/documents.

17. STUDY MONITORING

Monitoring will be performed by [redacted], the CRO qualified by Chiesi.

Monitoring will include on-site monitoring visits.

It is understood that the monitor(s) will contact and/or visit the Investigator/site before the study begins, regularly throughout the study, and after the study has been completed, and that they will be permitted to inspect the various study records (i.e. eCRFs, Investigator study files, and source data), provided that subject confidentiality is respected. Whenever needed (e.g. in case of emergency or sanitary situations), the monitoring CRO and the Sponsor may agree to convert planned on-site visits into telephone or video visits, or to postpone or completely cancel planned visits.

The purposes of these visits are:

- To verify that the safety and rights of participants are being protected;
- To assess the progress of the study;
- To review the compliance with the currently approved study protocol;

- To verify that the study is being conducted in accordance with ICH GCP, and all applicable regulatory requirements;
- To discuss any emergent problem;
- To validate that data entered in the eCRFs by authorised site personnel are accurate, complete and verifiable from the source documents (source data verification and source data review);
- To assess the status of drug storage, dispensing and retrieval.

Prior to each monitoring activity and upon prior notification by the monitoring activity, the Investigator or authorised staff will record all data generated since the last visit in the eCRFs. For on-site visits, 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. Similarly, in case of remote monitoring, the Investigator and/or study staff will be expected to be available for a remote contact with the monitor to answer questions and to provide any missing information.

It is possible that the investigational site may be audited by Sponsor personnel or regulatory national and/or international regulatory agencies during and/or after the study has been completed.

To ensure oversight and data (clinical and operational) reliability, the monitoring methodology for source data verification and reviewing could be a combination of:

- On-site monitoring;
- Remote monitoring.

The extent and nature of the monitoring will be determined on the basis of an assessment that considers the characteristics of the study, including the level of intervention of the study, its objectives and methodology, as well as the degree of the deviation of the interventions from normal clinical practice. The assessment is (regularly) reviewed (whenever an anticipated significant risk emerges).

18. QUALITY ASSURANCE

The Sponsor will implement processes to manage quality and oversight throughout all stages and activities of the study, based on data and processes identified as critical for the subjects' rights and safety as well as on data reliability and robustness.

Quality management will include system tools and procedures for data collection and processing, as well as the collection of information essential to decision-making.

The quality management system will use a risk-based approach as described in ICH E6, with:

- Critical Process and Data Identification;
- Risk Identification/Evaluation/Control/Communication and Review;
- Risk Reporting.

The Research and Development (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 GCP and the currently approved protocol.

19. INVESTIGATOR RESPONSIBILITIES

Investigator responsibilities are set out in the ICH Guideline for GCP and in the local regulations. Chiesi staff or an authorised representative will evaluate and approve all Investigators, who in turn will select their staff.

The Investigator must ensure that all staff assisting with the study are adequately informed about the protocol, amendments, and study treatments, as well as all study-related duties and functions, including obligations of confidentiality of Chiesi information.

The Investigator is to maintain a list of sub-Investigators and other appropriately qualified staff members or parties to whom he or she has delegated significant study-related duties. As well, the Investigator must ensure qualification of the service and integrity of any data generated.

The Investigator is responsible for keeping a record of all subjects who sign an ICF and are screened for entry into the study. Subjects who fail screening must have the reason(s) recorded in their source documents.

The Investigator or a designee must be available during monitoring visits to review data, resolve queries, and allow direct access to subject records (e.g. medical records, office charts, hospital charts, and study-related charts) for source data verification. The Investigator must ensure timely and accurate completion of eCRFs and queries.

The Investigator must notify the EC of SAEs or other significant safety findings as required by EC procedures.

The Investigator and his/her study staff are responsible for keeping confidential the information contained in the protocol and amendments (with the exception of the information provided by Chiesi on public registry websites). The Chiesi protocol, any protocol amendments, and information in the Investigator's brochure are not to be made publicly available (for example, on the Investigator's or institution's website) without express written approval by Chiesi.

The Investigator's responsibilities about the retention of records and documents are described in Section 27.

The Investigator must have oversight of the conduct of the study at the site and must adhere to the requirements of ICH guidelines, the EC, and all other applicable local regulations.

20. INSURANCE AND INDEMNITY

Chiesi holds and will maintain an adequate insurance policy covering damages arising out of its sponsored clinical research studies.

Chiesi will indemnify the Investigators and hold them harmless for claims for damages arising out of the study, in excess of those covered by their own professional liability insurance, providing that the drug was administered under their supervision (or that of a deputy) 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.

21. CONFIDENTIALITY

All study documents are provided by the Sponsor to the Investigator and his/her appointed staff in confidence. 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 that subjects' anonymity will be maintained. The Investigator will keep a separate list with at least each subject's study number, name, and date of birth. 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.

22. PERSONAL DATA AND DATA SECURITY

As a sponsor based in the European Union, Chiesi will collect and process subjects' data in accordance with the European General Data Protection Regulation (GDPR), and any applicable data protection laws and regulations. The personal data involved in the study will be processed in a pseudonymised form, in compliance with both GCP and data protection laws and regulations.

Chiesi is committed to abiding by all requirements foreseen by the applicable data protection laws and regulations, including processing subjects' data in compliance with data minimisation, transparency, and purpose limitation principles, and by implementing adequate technical and organisational safeguards, to ensure data security as provided under Article 32 of the GDPR. More specifically, all the Sponsor's employees are bound by confidentiality obligations set out in their employment contracts and the specific data processing instructions and training programs provided by the Sponsor. Therefore, the Sponsor's employees will not disclose or permit the disclosure of any of the information, including subjects' data and any other confidential, non-public, or proprietary information related to the clinical study protocol.

Access to subjects' data will be granted to the Sponsor's appointed employees through their personal credentials, solely for the purposes herein described and, on a need-to-know basis in compliance with the principles of purpose limitation and data minimisation. All study information, including subjects' personal data, will be processed and stored in an encrypted form to prevent its identification by non-authorised persons.

The Sponsor has implemented adequate cybersecurity measures, including best practices, software, hardware, and physical means to protect subject data and prevent any unexpected disclosure or threat that may affect the data. Chiesi will also conduct periodical cybersecurity assessments, such as penetration tests, to ensure that its systems are in line with the required security standards.

In compliance with the GDPR, Chiesi has in place a dedicated data breach management process to detect, mitigate, and, where applicable, notify the competent data protection authority of a data security breach affecting subject data. The process is coordinated by the Sponsor's data protection functions, including the appointed data protection officer (DPO) Office, which must be notified as soon as a data incident has been detected. After being notified, the DPO Office convenes a working group together with the cybersecurity function as well as with the business functions potentially affected by the incident. The working group, coordinated by the DPO office, is responsible for assessing the impact of the incident, and carries out activities necessary to mitigate any resulting problems. Based on the result of the evaluation of risks of the breach, the working group will determine whether it is necessary to notify the data protection authority and will proceed in compliance with the legal terms provided under Article 33 of the GDPR. In any case, the DPO Office keeps track of all the notified data incidents in a dedicated register.

The data breach management process described above will be initiated by the Sponsor upon notification by any parties involved in the study with whom Chiesi has entered into specific data processing agreements (e.g. CROs).

23. 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.

Reasons for the early closure of the investigational site, and therefore the early termination of the study by the Sponsor or an Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the EC or local health authorities, the Sponsor's procedures, or GCP guidelines;
- Inadequate or no recruitment (evaluated after a reasonable amount of time) of participants by the Investigator;
- Discontinuation of further study intervention development;
- Unjustifiable risk and/or toxicity in risk-benefit analysis, e.g. when AEs occur that are unknown to date with respect to their nature, severity, duration, or frequency in relation to the current established safety profile;
- New scientific evidence becomes available during the study that could affect subjects' safety;
- New insights are obtained from other clinical studies.

The Sponsor must submit a written notification to the concerned Regulatory Authority and EC providing the justification of premature ending or of the temporary halt.

24. CLINICAL STUDY REPORT

The clinical study report (CSR), including the statistical and clinical evaluations, shall be prepared and sent for agreement and signature to the Principal Investigator (for single-centre studies), when applicable.

The distribution of the CSR or CSR synopsis to all ECs and to the Competent Authority will follow local regulations.

25. DISSEMINATION OF CLINICAL STUDY DATA

In accordance with local legislative and applicable requirements on clinical studies, Chiesi will disclose protocol- and study-related information and the summary results of this clinical study in public registries (clinicaltrials.gov).

In accordance with Chiesi commitments on Clinical Trial Transparency (i.e. clinical study registration and posting of summary results, sharing the CSR synopsis, publication in scientific literature journals, and sharing of lay summary results), study information will be also publicly disclosed on a voluntary basis.

The Chiesi website www.chiesi.com contains the commitments on Clinical Trial Transparency and all the study information publicly disclosed.

25.1 SUMMARY OF RESULTS FOR STUDIES (TRIALS) CONDUCTED IN EUROPEAN UNION MEMBER STATES

Not applicable.

26. PUBLICATION POLICY

The results of this study may be published or presented at scientific meetings. If a publication is presented by the Investigator, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

Chiesi furthermore reserves the right to use such data for industrial purposes.

27. RECORD RETENTION

ICH E6 GCP guidelines require that essential documents of a clinical study be retained for at least two years after the final marketing approval in an ICH region, and until there are no pending or contemplated marketing applications in that region or until two years have elapsed since the formal interruption of the clinical development of the product under study.

Documents must be retained for a longer period if required by the applicable regulatory requirement(s).

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. In addition, all subjects' medical records and other source documentation will be kept for the maximum time permitted by the institution and in accordance with national laws.

No records may be destroyed at a clinical study site during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

It is the responsibility of the Sponsor to inform the Investigator of when these documents can be destroyed.

28. REFERENCES

[1] GBD 2015 Chronic Respiratory Disease Collaborators. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5(9):691-706.

[2] GBD Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Respir Med. 2020;8(6):585-596.

[3] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):e442.

[4] Vos T, Flaxman AD, Naghavi M, Lozano R, Michaud C, Ezzati M, Shibuya K, Salomon JA, et al. Years lived with disability (YLDs) for 1160 sequelae of 289 diseases and injuries 1990-2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet. 2012;380(9859):2163-2196.

[5] Croisant S. Epidemiology of asthma: prevalence and burden of disease. Adv Exp Med Biol. 2014;795:17-29.

[6] UN Environment Programme (UNEP) Annual Report 2014. https://www.unenvironment.org/resources/annual-report/unep-2014-annual-report. Accessed April 2021.

[7] The Montreal Protocol on Substances that Deplete the Ozone Layer. https://ozone.unep.org/treaties/montreal-protocol-substances-deplete-ozone-layer/text. Accessed April 2021.

[8] Myhre G, Shindell G, Bréon F-M, Collins W, Fuglestvedt J, Huang J, Koch D, Lamarque JD, et al. 2013: Anthropogenic and Natural Radiative Forcing. In: Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Stocker TF, Qin D, Plattner GK, Tignor M, Allen SK, Boschung J, Nauels A, Xia Y, Bex V, Midgley PM (eds.). Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. pp. 659-740.

[9] Jeswani H, Corr S, Azapagic A. Reducing carbon footprints of metered dose inhalers. Inhalation. 2017;11(6).

[10] Panigone S, Sandri F, Ferri R, Volpato A, Nudo E, Nicolini G. Environmental impact of inhalers for respiratory diseases: decreasing the carbon footprint while preserving patient-tailored treatment. BMJ Open Respir Res. 2020;7(1):e000571.

[11] Avella J, Kunaparaju N, Kumar S, Lehrer M, Zito SW, Barletta M. Uptake and distribution of the abused inhalant 1,1-difluoroethane in the rat. J Anal Toxicol. 2010;34(7):381-388.

[12] Ernstgård L, Sjögren B, Dekant W, Schmidt T, Johanson G. Uptake and disposition of 1,1-difluoroethane (HFC-152a) in humans. Toxicol Lett. 2012 February 25;209(1):21-29.

[13] Lovelace Biomedical. Clinical Study Report. A Phase I Dose HFA-152a Exposure Clinical Study in Healthy Male Volunteers (Protocol Number: Mex- HFA-152a). Version of 11 May 2021.

[14] European Medicines Agency. Committee for medicinal products for human use. Guideline on the requirements for clinical documentation for orally inhaled products (OIP) including the requirements for demonstration of therapeutic equivalence between two inhaled products for use in the treatment of asthma and chronic obstructive pulmonary disease (COPD) in adults and for use in the Treatment of asthma in children and adolescents. Document Reference: CPMP/EWP/4151/00 Rev. 1. London, 22 January 2009.

[15] Hasani A, Toms N, Agnew JE, Lloyd J, Dilworth JP. Mucociliary clearance in COPD can be increased by both a D2/β2 and a standard β2 agonists. Respiratory Medicine 2005;99:145–51.

[16] Restrepo RD. Inhaled adrenergics and anticholinergics in obstructive lung disease: do they enhance mucociliary clearance? Respir Care 2007;52:1159–73; discussion 1173-1175.

[17] Hasani A, Toms N, Agnew JE, Sarno M, Harrison AJ, Dilworth P. The Effect of Inhaled Tiotropium Bromide on Lung Mucociliary Clearance in Patients With COPD. Chest 2004;125:1726–34.

[18] Recommendations related to contraception and pregnancy testing in clinical trials (Clinical Trial Facilitation and Coordination Group. Final version 1.1 dd. 21/09/2020). 2020_09_HMA_CTFG_Contraception_guidance_Version_1.1_updated.pdf.

[19] Smittenaar CR, Petersen KA, Stewart K, Moitt N. Cancer incidence and mortality projections in the UK until 2035. Br J Cancer. 2016 Oct 25;115(9):1147-1155. doi: 10.1038/bjc.2016.304.

[20] Ahmad AS, Ormiston-Smith N, Sasieni PD. Trends in the lifetime risk of developing cancer in Great Britain: comparison of risk for those born from 1930 to 1960. Br J Cancer. 2015 Mar 3;112(5):943-7. doi: 10.1038/bjc.2014.606.

[21] Laube BL, Carson KA, Sharpless G, Paulin LM, Hansel NN. Mucociliary Clearance in Former Tobacco Smokers with Both Chronic Obstructive Pulmonary Disease and Chronic Bronchitis and the Effect of Roflumilast. Journal of Aerosol Medicine and Pulmonary Drug Delivery 2019;32:189–99.

[22] Fleming, John S., et al. 'Quantitative Assessment of Mucociliary Clearance in Smokers with Mild-to-Moderate Chronic Obstructive Pulmonary Disease and Chronic Bronchitis from Planar Radionuclide Imaging Using the Change in Penetration Index'. Journal of Aerosol Medicine and Pulmonary Drug Delivery, vol. 32, no. 4, Aug. 2019, pp. 175–88. PubMed, https://doi.org/10.1089/jamp.2017.1441.

[23] EudraLex. The Rules Governing Medicinal Products in the European Union. Volume 4 - EU Guidelines to Good Manufacturing Practice. Medicinal Products for Human and Veterinary Use. Annex 13 – Manufacture of Investigation Medicinal Products. 2010. https://ec.europa.eu/health/sites/health/files/files/eudralex/vol-4/2009_06_annex13.pdf. Accessed March 2021.

[24] COVID-19 Rapid guideline: arranging planned care in hospitals and diagnostic services. National Institute for Health and Care Excellence (NICE) guideline NG179. 2020 July 27. Overview | COVID-19 rapid guideline: arranging planned care in hospitals and diagnostic services | Guidance | NICE. Accessed February 2022.

[25] Graham BL, Steenbruggen I, Miller MR, Barjaktarevic IZ, Cooper BG, Hall GL, Hallstrand TS, Kaminsky DA, et al. Standardization of Spirometry 2019 Update. An Official American Thoracic Society and European Respiratory Society Technical Statement. Am J Respir Crit Care Med. 2019;200(8):e70-e88.

[26] Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, Enright PL, Hankinson JL, et al; ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-1343.

APPENDIX 1
Approval of the protocol by the Principal Investigator

OPEN-LABEL, RANDOMISED, CONTROLLED, 2-WAY CROSS-OVER STUDY TO ASSESS THE EFFECT OF MULTIPLE DOSES OF THE NEW HFA-152a PROPELLANT VERSUS THE MARKETED HFA-134a PROPELLANT ON MUCOCILIARY CLEARANCE IN HEALTHY VOLUNTEERS.

Product:

Test product: Placebo HFA-152a propellant

Reference product: Placebo HFA-134a propellant

Pharmaceutical Form:

Test product: pressurised metered dose inhaler (pMDI) with HFA-152a

Reference product: pressurised metered dose inhaler (pMDI) with HFA-134a

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 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: _____, MBChB

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, postmenopausal state 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 postmenopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy.

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 postmenopausal 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.

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 (oestrogen 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 [3];
- Intrauterine device (IUD) [3];
- Intrauterine hormone-releasing system (IUS) [3];
- Bilateral tubal occlusion [3];
- Vasectomised partner [1,3];
- 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.

[3] Methods with lower user dependency.

Reference: Recommendations related to contraception and pregnancy testing in clinical trials (Clinical Trial Facilitation and Coordination Group. Final version 1.1 dd. 21/09/2020).