Sponsor:
**AQUILON PHARMACEUTICALS S.A.**
Quai de la Boverie, 59
4020 Liège, Belgium

# BOREAS

## CLINICAL TRIAL PROTOCOL

**A prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial to assess the efficacy, safety and pharmacokinetics of a budesonide inhalation solution (AQ001S) compared to a budesonide inhalation suspension (comparator) in adults with mild asthma**

**Clinical trial code:** BOREAS
**Phase:** 1/2a
**Investigational Medicinal Product under investigation:** AQ001S 0.125 mg/ml (budesonide 0.125 mg/ml inhalation solution)
**Indication:** Asthma
**EudraCT**: 2019-002849-38

**Version**: 3.0 (final)
**Date: 15-JUN-2021**
**Replaces version:** 2.0
**Sponsor:** Aquilon Pharmaceuticals S.A.

# 1. CLINICAL TRIAL SUMMARY INFORMATION

| | |
|---|---|
| **Clinical trial title** | A prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial to assess the efficacy, safety and pharmacokinetics of a budesonide inhalation solution (AQ001S) compared to a budesonide inhalation suspension (comparator) in adults with mild asthma. |
| **Investigational medicinal product (IMP) under investigation** | AQ001S 0.125 mg/ml (budesonide 0.125 mg/ml inhalation solution) |
| **Indication** | Maintenance treatment of mild asthma |
| **Clinical trial design** | Prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial |
| **Sponsor** | Aquilon Pharmaceuticals S.A. (Aquilon)<br>Quai de la Boverie, 59<br>4020 Liège<br>BELGIUM<br>Phone: +32 (0) 4 229 28 00 |
| **Clinical trial code** | BOREAS |
| **EudraCT Number** | 2019-002849-38 |
| **Development phase** | Phase 1/2a |
| **Estimated clinical start** | June 2021 |
| **Estimated trial duration** | 6 months |
| **Date of Protocol** | 15-JUN-2021 |
| **Version** | 3.0 (final) |
| **Sponsor signatory** | Name:<br>Title: Clinical and Project Leader<br>Phone :<br>Mobile :<br>Email: |
| **Sponsor's medical advisor** | Name:<br>Title: Physician<br>Affiliation:<br>Mobile:<br>Email: |
| **Sponsor's medical expert** | Name:<br>Title: Physician, Professor<br>Affiliation:<br>Phone:<br>Mobile:<br>Email: |
| **Safety Monitor for the trial** | Name:<br>Title: Medical Director<br>Affiliation:<br>Mobile:<br>Email: |
| **Pharmacovigilance Manager for the trial** | Name:<br>Title: Pharmacovigilance Manager<br>Affiliation:<br>Mobile :<br>Email: |

| | |
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| **Principal Investigator and qualified physician responsible for all trial-site related medical decisions** | Dr.<br>*Address: see trial site*<br>Phone :<br>Mobile :<br>Email: |
| **Trial site** | |
| **Contract Research Organization (CRO) in charge of the clinical project management and monitoring** | Phone: |
| **Manufacturing of IMP under investigation** | Phone : |
| **Packaging and labelling of IMP under investigation, IMP used as a comparator and auxiliary medicinal product used in the trial** | Phone: |
| **Local Safety Laboratory (hematology, biochemistry and urinalysis)** | Phone : |
| **Local Safety Laboratory (urinary cortisol)** | Phone: |
| **Bioanalysis laboratory** | Phone : |

## 2. SYNOPSIS

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|---|---|
| **Clinical trial code** | BOREAS |
| **Sponsor** | Aquilon Pharmaceuticals S.A. |
| **Investigational medicinal product (IMP) under investigation** | AQ001S 0.125 mg/ml (budesonide 0.125 mg/ml inhalation solution) |
| **Title** | A prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial to assess the efficacy, safety and pharmacokinetics of a budesonide inhalation solution (AQ001S) compared to a budesonide inhalation suspension (comparator) in adults with mild asthma. |
| **Phase** | Phase 1/2a<br>First-In-Human<br>Proof-Of-Concept |
| **Primary Objective - Efficacy** | To compare the efficacy, i.e. the bronchoprotection of AQ001S 0.125 mg/ml with the comparator. |
| **Safety Objective** | To assess the safety of AQ001S 0.125 mg/ml. |
| **Secondary Objectives** | 1. To compare the pharmacokinetics (PK) of AQ001S 0.125 mg/ml with the comparator<br>2. To compare the secondary Efficacy/Pharmacodynamics (PD) of AQ001S 0.125 mg/ml with the comparator. |
| **Trial design** | Prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial |
| **Trial population** | Adults with mild asthma (18 to 65 years old), who are 'inhaled corticosteroid (ICS)'-naïve for minimum 60 days at Screening Visit.<br>A total of 24 subjects will be recruited into the clinical trial. Considering potential dropouts, a total of 20 subjects are anticipated to complete the clinical trial. |
| **Trial location** | This clinical trial will be conducted in 1 trial site in Belgium. |
| **Main Inclusion Criteria** | 1. Reliable subjects who are willing to be available for the duration of the clinical trial and willing to comply with clinical trial procedures.<br>2. Subjects who have the ability to understand the requirements of the clinical trial.<br>3. Subjects who have given written informed consent.<br>4. Subjects aged between 18 and 65 years, inclusive.<br>5. Body mass index between 18.5 and 29 $kg/m^2$.<br>6. Documented clinical diagnosis of stable, persistent, asthma for at least 3 months, i.e.:<br>• for whom forced expiratory volume in one second ($FEV_1$) ≥ 70% of predicted, and<br>• treated with as-needed reliever medication (short-acting beta2-agonist-containing medication) only.<br>7. Subjects who are ICS-naïve for minimum 60 days at Screening Visit.<br>8. Positive methacholine (MCh) challenge test (concentration of MCh provoking an $FEV_1$ fall of 20% [PC20] < 8 mg/ml or dose of MCh provoking an $FEV_1$ fall of 20% [PD20] < 0.2 mg) in the last year.<br>9. Post-bronchodilator $FEV_1$ at least 80% of the predicted, documented in the last year.<br>10. Clinical laboratory test results, 12-lead electrocardiogram (ECG), blood pressure and heart rate (supine) within normal reference range or judged to be not clinically significant by the Investigator.<br>11. Female subjects of childbearing potential should have a negative pregnancy test at Screening Visit.<br>12. Female subjects of childbearing potential using a highly effective method of contraception (i.e., pregnancy rate of < 1% per year) on a stable regimen, for at least 28 days, and pursuing this contraception during the trial and for 28 days after the last administration of the IMP. The highly effective methods of contraception must be one of the following: |

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| | combined estrogen and progestogen hormonal contraception with inhibition of ovulation, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner, or agreement on continuous abstinence from heterosexual intercourse. |
| **Main Exclusion criteria** | 1. Current smokers or recent (< 8 weeks) ex-smokers or ex-smokers if > 10 pack-years.<br>2. Pregnant or breastfeeding female subjects.<br>3. Inability to carry out pulmonary function testing.<br>4. $FEV_1$ < 70%.<br>5. History of near-fatal asthma and/or intensive care unit admission for asthma symptoms.<br>6. Exacerbations of asthma requiring oral steroids, hospitalization or change in asthma treatment in the previous three months.<br>7. Evidence of symptomatic chronic or acute respiratory infection in the previous 8 weeks.<br>8. Diagnosis of chronic obstructive pulmonary disease (COPD) or bronchiectasis.<br>9. Pulmonary malformations, tuberculosis, cystic fibrosis.<br>10. History of hypersensitivity or existing contraindication to budesonide or any other IMP ingredients.<br>11. Untreated oral candidiasis.<br>12. Immunosuppressive treatment, including systemic corticosteroids (e.g., oral, parenteral, ocular, nasal), within 28 days before Screening Visit.<br>13. Use of ICS within 60 days before Screening Visit.<br>14. Use of anti-leukotrienes, immunoglobulins, beta-blockers, digitalis, amiodarone, antifungals, macrolides, antidepressants, monoamine oxidase inhibitors, antiretroviral drugs, cholinesterase inhibitors, histamine, theophylline, non-steroidal anti-inflammatory drugs, anticholinergic drugs, neuroleptics, curariform drugs, antihistaminic (anti-$H_1$) drugs, calcium channel blockers, long acting beta2-antagonists, mast cell stabilizers (e.g. natrium cromoglycate).<br>15. History of alcohol or drug abuse.<br>16. Unstable or life-threatening cardiac disease: subjects with any of the following would be excluded:<br>• Myocardial infarction or unstable angina in the 6 months before Screening Visit,<br>• Unstable or life-threatening cardiac arrythmia requiring intervention in the 3 months prior to Screening Visit,<br>• New York Heart Association class IV Heart failure.<br>17. History or presence of prolonged QT interval (> 470 ms), or any other clinically significant ECG abnormalities as judged by the Investigator based on 12-lead ECG recordings at Screening Visit.<br>18. Diabetes mellitus.<br>19. Neuropsychiatric diseases.<br>20. Clinically relevant laboratory abnormalities at Screening Visit.<br>21. Blood or plasma donation within 30 days prior to Screening Visit.<br>22. History or presence of malignancy of any system organ class (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years prior to Screening Visit, regardless of whether there is evidence of local recurrence or metastases.<br>23. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the subject in case of participation in the clinical trial.<br>24. History or presence of any other clinically relevant disease of any major system organ class (e.g. cardiovascular, pulmonary, renal, hepatic, |

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| | gastrointestinal, reproductive, endocrinological, neurological, psychiatric or orthopedic disease) as judged by the Investigator.<br>25. Human immunodeficiency virus (HIV) and SARS-CoV-2 infections.<br>26. Subjects who participated in an investigational trial within the 12 weeks prior to the start of the trial. |
| **Investigational Medicinal Products (IMPs)** | IMP under investigation:<br>• AQ001S 0.125 mg/ml is a budesonide inhalation solution administered by nebulization once daily.<br>Comparator:<br>• Budesonide 0.125 mg/ml is a budesonide inhalation suspension administered by nebulization once daily. |
| **Duration (per subject)** | 77 to 85 days from Screening Visit, including two treatment periods of 29 (+2) days and one washout period of 14 (+2) days, between the treatment periods.<br>In case the subject is taking or has taken ICS within the last 60 days at the time of the Pre-Trial Visit (the visit before or combined to the Screening Visit), the total duration might be a maximum of 147 days from Pre-Trial Visit. |
| **Administration of the IMPs** | - At trial site on D0, D1 and D28 (+2) of each treatment period.<br>- At home in the morning by a study nurse from D2 up to the penultimate day of each treatment period. |
| **Primary efficacy endpoint** | Bronchoprotection will be assessed by PC20, as determined by MCh challenge test.<br>The primary efficacy endpoint will be the change from baseline in PC20 after each treatment period (baseline is defined at Visit 1). |
| **Safety endpoints** | The safety will be evaluated collecting the following information:<br>• Adverse events and serious adverse events, including asthma exacerbations<br>• General tolerability: vital signs, ECG, physical examination<br>• Laboratory parameters: hematology, biochemistry and urinalysis<br>• Local tolerability:<br>o Increased bronchial irritability<br>o Paradoxical bronchospasm<br>o Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection)<br>• $FEV_1$ and forced vital capacity (FVC), measured by spirometry<br>• Assessment of hypothalamic pituitary adrenocortical (HPA) axis function: urinary cortisol/creatinine ratio in first waking urine |
| **Pharmacokinetic endpoints** | The PK endpoint, i.e. the PK profile of budesonide in plasma, will be evaluated through the following PK parameters, calculated for each treatment period:<br>• Start of treatment period:<br>o single dose 24-hour PK parameters at the time of the first scheduled IMP administration (16 time points): $C_{max}$, $t_{max}$, $C_{last}$, $t_{last}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$, CL/F, V/F, $\lambda_z$ and $t_{1/2z}$.<br>• End of treatment period:<br>o abridged PK at the time of the last scheduled IMP administration (7 time points): $C_{through}$, $C_{max}$, $t_{max}$, $C_{last}$, $t_{last}$, and $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$. |
| **Secondary Efficacy/ Pharmacodynamic endpoints** | The following secondary efficacy/PD parameters will be evaluated:<br>• Symptom scores recording: day- and night-time asthma symptom scoring and validated asthma-specific questionnaires (included in trial subject diary).<br>• Use of as-needed reliever medication (as reported in trial subject diary).<br>• Airway inflammation PD biomarkers: fractional concentration of exhaled nitric oxide (FeNO) and blood eosinophil count.<br>• $FEV_1$ and forced inspiratory vital capacity (FIVC), measured by spirometry. |
| **Statistical methods** | For primary efficacy analysis, the mean PC20 changes from baseline between AQ001S 0.125 mg/ml and the comparator will be compared by analysis of covariance (ANCOVA) for crossover design. A p-value will be calculated for the |

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| | difference of the parameter between AQ001S 0.125 mg/ml and comparator. If this p-value is significant and the difference prefers AQ001S 0.125 mg/ml, ***superiority*** is shown.<br>Additionally, an adjusted 95%-confidence interval for the mean difference between AQ001S 0.125 mg/ml and the comparator will be obtained by the ANCOVA.<br>For the safety analysis, the treatment groups will be compared in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics (at least number of observations, mean, standard deviation, median, minimum and maximum) will be used to analyze continuous (quantitative) data. |

## 3. FLOW CHART OF TRIAL PROCEDURES

| Procedures / Periods | Screening | | Treatment period 1 (TP1) | | Washout | Treatment period 2 (TP2) | |
|---|---|---|---|---|---|---|---|
| Visits[1] | Pre-Trial Visit | Screening Visit | Visit 1 (Randomization and Baseline) | Visit 2 | | Visit 3 | Visit 4 (End-of-Trial)[2] |
| Timing of visits (treatment day) | Between D-67 (-2) and D-5[3] | Between D-7 and D-5 | TP1 D0 and D1 | TP1 D28 (+2) | 14 (+2) days | TP2 D0 and D1 | TP2 D28 (+2) |
| Informed consent | X | | | | | | |
| Subject Screening ID assignment | X | | | | | | |
| Inclusion/exclusion criteria | | X | | | | | |
| Demographics | | X | | | | | |
| Medical and surgical history | | X | | | | | |
| Respiratory/asthma history | | X | | | | | |
| Medication history (prior medication)[4] | | X | X | | | | |
| Concomitant medication[5] | | | X | X | | X | X |
| Physical and clinical examination[6] | | X | X | X | | X | X |
| Serum pregnancy test | | X | | | | | |
| Urine pregnancy test | | | X | | | | |

[1] The procedures planned for all visits (except the Pre-Trial Visit) should be performed at least 4 hours after the subject has taken any as-needed reliever medication. The subject should come fasted (for at least 8 hours).
[2] In case of early termination or withdrawal of the subject, the procedures listed in Visit 4 will be performed according to the subject's health condition at the discretion of the Investigator.
[3] Pre-Trial Visit might be performed at the same time as the Screening Visit if the ICS washout period is ≥ 60 days and if the subject has been fasting for at least the last 8 hours. Screening Visit should be planned as soon as all these conditions are met, so at maximum 60 (+2) days after the Pre-Trial Visit.
[4] Prior medications taken in the 3 months prior to Screening Visit, including respiratory medications and especially the use of as-needed reliever medication (SABA-containing medication) and any other asthma medications.
[5] Any medications taken from the first IMP administration at Visit 1 will be recorded by the Investigator as concomitant medications.
[6] Weight, height (only measured at Screening Visit), body mass index, oropharyngeal examination by the Investigator (physician) and COVID-19 symptoms assessments.

| Procedures / Periods | Screening | | Treatment period 1 (TP1) | | Washout | Treatment period 2 (TP2) | |
|---|---|---|---|---|---|---|---|
| Visits[1] | Pre-Trial Visit | Screening Visit | Visit 1 (Randomi-zation and Baseline) | Visit 2 | | Visit 3 | Visit 4 (End-of-Trial)[2] |
| Timing of visits (treatment day) | Between D-67 (-2) and D-5[3] | Between D-7 and D-5 | TP1 D0 and D1 | TP1 D28 (+2) | 14 (+2) days | TP2 D0 and D1 | TP2 D28 (+2) |
| First waking urine collection for cortisol/creatinine ratio determination | | | X[7] | X[7] | | X[7] | X[7] |
| Vital Signs | | X | X | X | | X | X |
| 12-lead ECG[8] | | X | | X | | | X |
| Fasting (8-hour) blood and urine sampling for hematology, biochemistry and urinalysis | | X | X[7] | X[7] | | X[7] | X[7] |
| Spirometry[9] | | X | X | X | | X | X |
| FeNO measurement[10] | | | X | X | | | X |
| Blood eosinophil count[11] | | | X | X | | | X |
| MCh challenge test (PC20 determination) | | (X)[12] | X | X | | | X |
| Administration of as-needed reliever medication (SABA)[13] | | (X) | (X) | (X) | | | (X) |
| Randomization and Randomization ID assignment | | | X | | | | |
| On-site IMP administration | | | X[14] | X[15] | | X[14] | X[15] |

[7] Blood and urine collections will be combined with those necessary for the blood PK sampling and eosinophil count, and for the urinary cortisol/creatinine ratio determination, i.e. in the first waking urine.

[8] The single 12-lead ECG will be recorded while the subject is in resting position (supine) for at least 5 minutes.

[9] Screening Visit: Measurement of $FEV_1$; Visits 1, 2 and 4: Measurements of $FEV_1$, FVC and FIVC before and after MCh challenge test; Visit 3: Measurements of $FEV_1$ and FVC.

[10] FeNO measurement will be performed before MCh challenge.

[11] Blood eosinophil count as a PD parameter, calculated at Visits 1, 2 and 4 from the hematology results, will be performed during PK sampling before MCh challenge.

[12] If not performed in the last year and if $FEV_1 \geq 70\%$ (for inclusion: PC20 < 8 mg/ml or PD20 < 0.2 mg).

[13] After each MCh challenge test, if $FEV_1 < 70\%$ or presence of asthma clinical symptoms (e.g. dyspnea, cough, wheezing, bronchospasm, etc.), administer as-needed reliever medication (SABA).

[14] Administration of the D0 and D1 doses of the treatment period.

[15] Administration of the last dose of the treatment period.

| Procedures / Periods | Screening | | Treatment period 1 (TP1) | | Washout | Treatment period 2 (TP2) | |
|---|---|---|---|---|---|---|---|
| Visits[1] | Pre-Trial Visit | Screening Visit | Visit 1 (Randomization and Baseline) | Visit 2 | | Visit 3 | Visit 4 (End-of-Trial)[2] |
| Timing of visits (treatment day) | Between D-67 (-2) and D-5[3] | Between D-7 and D-5 | TP1 D0 and D1 | TP1 D28 (+2) | 14 (+2) days | TP2 D0 and D1 | TP2 D28 (+2) |
| PK sampling | | | X[16] | X[17] | | X[16] | X[17] |
| Overnight stay of subject at trial site | | | X | | | X | |
| IMP dispensing | | | X | | | X | |
| Return of IMP and drug accountability | | | | X | | | X |
| Subject diary dispensing[18] | | X | | | | | |
| Subject diary return and review | | | X | X | | X | X |
| (Serious) Adverse Events[19] assessment | | | X | X | | X | X |
| Local tolerability[20] | | | X | X | | X | X |
| Subsequent therapy counselling | | | | | | | X |

[16] Sixteen (16) blood samples over 24 hours (sampling at the clinical site), including 1 pre-dose before IMP administration and 15 post-dose at , , , 20, 30, 45, 60, 90, 120, 180, 240, 360 minutes and 10, 18, 24 hours after IMP administration (full single 24-hour PK).

[17] Seven (7) blood samples taken over 6 hours (sampling at the clinical site on D28[+2]), including 1 pre-dose before IMP administration and 6 post-dose at , , , 120, 240 and 360 minutes after IMP administration (abridged PK).

[18] Trial subject diaries include daily day- and night-time asthma symptom questionnaires, weekly ACQ-5 and monthly ACT questionnaires, information about as-needed reliever medication (SABA) and other concomitant medication use.

[19] Whenever a (serious) adverse event takes place.

[20] Through physical and clinical examination: increased bronchial irritability, paradoxical bronchospasm, oropharyngeal examination by the Investigator (physician) (e.g. vocal cord myopathy, oral fungal infection).

ACQ = asthma control questionnaire; ACT = asthma control test; D = treatment day, D0 being the first IMP administration of the treatment period; ECG = electrocardiogram; FeNO = fractional concentration of exhaled nitric oxide; $FEV_1$= forced expiratory volume at 1 second; FIVC = forced inspiratory vital capacity; FVC = forced vital capacity; ICS = inhaled corticosteroid; ID = identification (number); IMP = investigational medicinal product; MCh = methacholine (chloride); PC20 = concentration of MCh provoking an $FEV_1$ fall of 20%; PD = pharmacodynamics; PD20 = dose of MCh provoking an $FEV_1$ fall of 20%; PK = pharmacokinetics; SABA = short-acting beta2-agonist

# 4. TABLE OF CONTENTS, FIGURES AND TABLES

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## FIGURES



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**APPENDICES**

## 5. ACRONYMS AND ABBREVIATIONS

| | |
|---|---|
| ACT | Asthma Control Test |
| ACQ | Asthma Control Questionnaire |
| AE | Adverse Event |
| ANCOVA | Analysis of Covariance |
| AR | Adverse Reaction |
| AxMP | Auxiliary Medicinal Product |
| BE | Belgium |
| COPD | Chronic Obstructive Pulmonary Disease |
| CRO | Contract Research Organization |
| (e)CRF | (electronic) Case Report Form |
| ECG | Electrocardiogram |
| EDC | Electronic Data Capture |
| EFF | Efficacy population |
| FeNO | Fractional exhaled Nitric Oxide |
| $FEV_1$ | Forced Expiratory Volume in 1 second |
| FIH | First-In-Human |
| FIVC | Forced Inspiratory Vital Capacity |
| FVC | Forced Vital Capacity |
| GCP | Good Clinical Practice |
| GDPR | Global Data Protection Regulation |
| GINA | Global Initiative for Asthma |
| HIV | Human Immunodeficiency Virus |
| HPA | Hypothalamic Pituitary Adrenocortical |
| HP-Betadex | 2-hydroxypropyl-β-cyclodextrin |
| IB | Investigator's Brochure |
| ICS | Inhaled Corticosteroid |
| ID | Identification (number) |
| IEC | Independent Ethics Committee |
| IFU | Instructions For Use |
| IMP | Investigational Medicinal Product |
| IRB | Institutional Review Board |
| LABA | Long-Acting Beta2-Agonist |
| LAMA | Long-Acting Muscarinic Antagonist |
| MCh | Methacholine (chloride) |
| ms | Millisecond(s) |
| PC20 | Concentration of methacholine provoking an $FEV_1$ fall of 20% |
| PD | Pharmacodynamics |
| PD20 | Dose of methacholine provoking an $FEV_1$ fall of 20% |
| pH | hydrogen Potential |
| PK | Pharmacokinetics |

| | |
|---|---|
| POC | Proof-Of-Concept |
| QD | *quaque die* (once daily) |
| SABA | Short-Acting Beta2-Agonist |
| SAE | Serious Adverse Event |
| SAF | Safety population |
| SAP | Statistical Analysis Plan |
| SARS-CoV-2 | Severe acute respiratory syndrome coronavirus 2 |
| SD | Standard Deviation |
| SDV | Source Data Verification |
| SmPC | Summary of Product Characteristics |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| US(A) | United States (of America) |

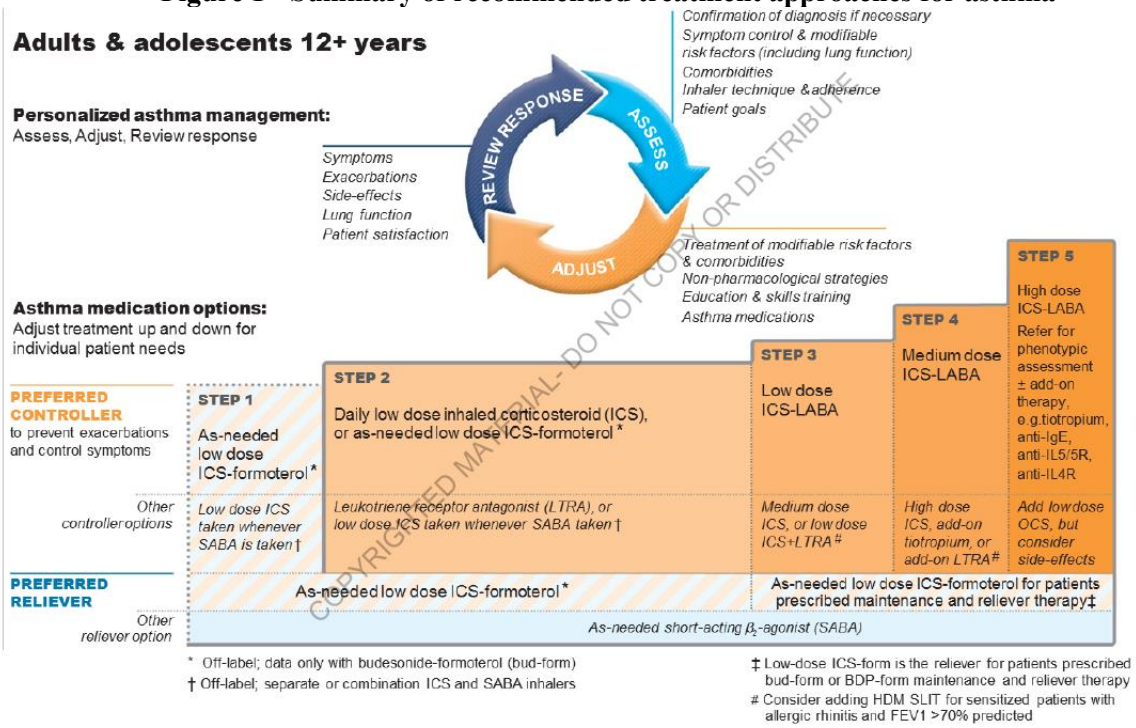
# 6. INTRODUCTION

## 6.1. Background Information

Asthma is a chronic inflammation of the airways that causes coughing, chest tightness, wheezing or shortness of breath. The disease is mainly due to genetic (inherited) and/or environmental factors such as allergens, air pollutants and irritants like smoke. Respiratory infections and physical activity can also trigger asthma [1].

To improve asthma prevention and management, the "Global Initiative for Asthma" (GINA), a network of public health officials and health care professionals created by the American National Lung Institute and the World Health Organization, have released the GINA strategy, i.e. a global consensus on how to treat asthma (last version: 2019) [2]. Every practitioner worldwide is invited to follow the recommended step-up approaches (summarized in Figure 1) to select the most appropriate treatment according to patient's disease severity, disease control and age category. In brief, asthma maintenance treatment is mainly based on lung inflammation control by anti-inflammatory drugs, in particular inhaled corticosteroids (ICS), and on airway opening with bronchodilator drugs (mainly long-acting beta2-agonists [LABAs]) from low to high doses according to the stage of the disease. In case of acute crisis, low dose ICS and LABA combinations are now the preferred as-needed reliever medication beside the traditional short-acting beta2-agonist (SABA) bronchodilators. When the disease is very severe, other types of maintenance treatment should be added, i.e. long-acting muscarinic antagonists (LAMAs) bronchodilators or oral corticosteroids.

**Figure 1 - Summary of recommended treatment approaches for asthma**






Source: GINA report 2019 [2]

BDP: beclometasone dipropionate; bud: budesonide; FEV1= forced expiratory volume at 1 second; form: formoterol; GINA: Global Initiative for Asthma; HDM SLIT: house dust mite sublingual immunotherapy; ICS: inhaled corticosteroid; IgE: immunoglobulin E; anti-IL(R): anti-interleukin (receptor); LABA: long-acting beta2-agonist; LTRA: leukotriene receptor antagonist; OCS: oral corticosteroid; SABA: short-acting beta2-agonist.

The majority of these treatments are administered by inhalation. Despite the established efficacy of ICS in asthma treatment, the lung deposition of currently marketed reference therapies is low and

heterogeneous. In case of administration by nebulization, on average, only 10% of the dose released from the traditional jet nebulizers will reach the site of action, i.e. deep lung areas [3–5]. This leads to decreased treatment efficacy and higher ICS doses are usually prescribed to compensate these drawbacks. These higher ICS doses increase the risk of local and systemic dose-dependent side effects. Side effects of ICS, in particular local ones such as hoarseness, throat irritation and oral fungal infection, are common in clinical practice due to the usual chronic administration of ICS [2,6,7].
Budesonide is an ICS approved since 1981 [8] and marketed in liquid and powder formulations intended for inhalation to treat asthma (Pulmicort Respules®, Turbohaler®, Flexhaler® and generics) [9–15]. Budesonide is recommended by the international and national regulatory authorities as a first-line maintenance therapy for asthma management in adult and pediatric patients and its safety is well established [16–20].
Currently, the only budesonide liquid formulation for nebulization available on the market is an aqueous suspension (Pulmicort Respules® and generics), i.e. a heterogenous mixture containing solid particles, because of budesonide's lipophilic properties and low solubility in water [10,12,14]. Suspensions can only be nebulized with traditional jet nebulizers [10,11,14,21–23]. The more recent nebulizers, especially the vibrating mesh nebulizers, which offer several benefits compared to the jet ones (e.g. hand-held, battery-driven, shorter time of administration) are not compatible with suspensions because of their specific technologies [24–26] and can only be used with aqueous solutions, i.e. homogenous mixtures where particles have been totally dissolved. Hence patients treated with suspension formulations, e.g. budesonide suspensions, cannot benefit from the advantages offered by more recent nebulizers.
The low solubility of budesonide can therefore create technical difficulties and, consequently, jeopardize treatment efficacy and safety, which could have been improved through the use of these more recent nebulizers.

Aquilon uses 2-hydroxypropyl-β-cyclodextrin (HP-Betadex) as a new excipient for inhaled drugs to solubilize budesonide, as both molecules form together a molecular inclusion complex. The resulting formulation is a homogenous budesonide inhalation solution (AQ001S) [27–29]. HP-Betadex is a cyclic oligosaccharide able to form water-soluble inclusion complexes of lipophilic water-insoluble drugs. HP-Betadex is used to this intent in marketed oral, ocular, parenteral and rectal products in Europe and in the US (on the market: Sporanox®, Indocollyre®; withdrawn: Propulsid®, Mitozytrex®). Moreover, an orphan drug designation has been issued since 2009 for the compassionate use of HP-Betadex at high dosages (intrathecal and intracerebroventricular administration) [30–34]. However, the use of HP-Betadex in inhaled drugs is new. To assess its safety and potential activity when administered by inhalation, HP-Betadex has been tested in 2 clinical trials (phase 1 and phase 2) by inhalation. This study showed that HP-Betadex administered by inhalation (nebulization) in multiple doses is proved to be safe and well-tolerated [35,36]. Preclinical toxicology studies on HP-Betadex and AQ001S in rats did not point out any safety concerns for the intended clinical dose strengths (also further described and summarized in AQ001S' Investigator's Brochure [IB]).

*In vivo* Proof-Of-Concept (POC) studies performed by Aquilon in experimental murine asthma models have shown that intranasal administration of AQ001S has higher efficacy in reducing peribronchial allergen-induced inflammation than a budesonide suspension given intranasally at the same dose [28,37]. Indeed, AQ001S showed the same efficacy against several asthma outcomes as Pulmicort Respules®, but at lower budesonide doses. At the same budesonide doses, AQ001S efficacy against asthma symptoms was higher than Pulmicort Respules®. Hence, budesonide complexation by HP-Betadex increases budesonide efficacy in these animal models of asthma. This could lead, in humans, to a reduction of the budesonide dose required to obtain efficacy against asthma symptoms, and therefore to a decrease of patient exposure to ICS.

These promising data open new therapeutic perspectives in asthma management, i.e.:
- the possibility to use AQ001S with any nebulizer device in contrast to budesonide suspensions,

- a decrease of patient exposure to ICS and thus to ICS-related adverse events (AEs) due to the lower budesonide dose in AQ001S needed to treat asthma in comparison to budesonide suspensions.

AQ001S is meant to be administered by inhalation via nebulizer and its target indication is the maintenance treatment of asthma.

## 6.2. Rationale for Conducting the Clinical Trial

In the framework of a Phase 1/2a clinical program on AQ001S, Aquilon is planning to perform a phase 1/2a First-In-Human (FIH) POC clinical trial, comparing AQ001S at the dose of 0.125 mg/ml (AQ001S 0.125 mg/ml) with a budesonide inhalation suspension at 0.125 mg/ml (comparator, Budesonide 0.125 mg/ml, Section 8.3.2). In this clinical trial named BOREAS, mild asthmatic adult subjects will receive the same dose of AQ001S and of the comparator, i.e. 0.125 mg of budesonide daily. Both investigational medicinal products (IMPs) will be administered for a short treatment duration (total of 29 [+2] days) with a conventional jet nebulizer, in a crossover design. The goal is to establish the POC of AQ001S by assessing and comparing its efficacy, safety and pharmacokinetics (PK) to those of the comparator. Based on the results of the nonclinical data cited in Section 6.1, it is hypothesized that AQ001S 0.125 mg/ml will show a higher efficacy in treating asthma than the comparator used at the same dose.

The recommended doses of budesonide inhalation suspension (original product: Pulmicort Respules®; generic: Budesonide ) for treatment initiation and maintenance in adults and in children in Belgium are presented in Table 1 [11,38] and slightly differ between the original and generic products. In comparison with the doses listed in Table 1, a dose of 0.125 mg budesonide once daily (QD) is low and supposed to have very low efficacy in asthma control, at least when administered as a suspension.

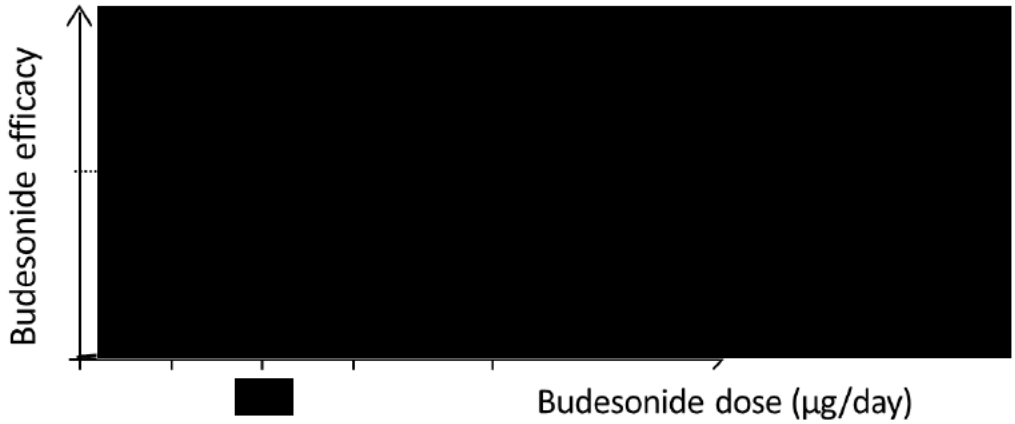
**Table 1 - Recommended doses of budesonide inhalation suspension for asthma treatment in**

| Reference | Formulation | Budesonide dose (mg/day) | |
|---|---|---|---|
| | | Adults, elderly and adolescents (≥ 12 years old) | Children (3 months to 12 years old) |
| **Recommended starting dose** | Budesonide inhalation suspension (Pulmicort Respules®) | 1-2 | 0.25-0.5 |
| **Recommended maintenance dose** | Budesonide inhalation suspension (Budesonide ) | 0.5-2 | 0.25-1 |
| | Budesonide inhalation suspension (Pulmicort Respules®) | 0.5-4[a] | 0.25-2 |
| **BOREAS clinical trial** | Budesonide inhalation solution (AQ001S 0.125 mg/ml) | 0.125 | - |
| | Budesonide 0.125 mg/ml (comparator) | 0.125 | - |

[a] doses can be increased further in severe cases. Adapted from [11,14].

This low budesonide dose chosen for the BOREAS clinical trial was selected to observe therapeutic effects and PD (Figure 2).

**Figure 2 - Simulation of the response curves of AQ001S (budesonide inhalation solution, extrapolated from nonclinical data) and of the comparator (suspension reference, extrapolated from the literature)**




In sum, in the BOREAS clinical trial, the efficacy, safety and PK of AQ001S 0.125 mg/ml and the comparator will be assessed and compared. Each subject will receive both IMPs for 29 (+2) days in a crossover design. Both AQ001S 0.125 mg/ml and the comparator will be administered by inhalation and particularly by nebulization as recommended for liquid formulations. A dose of 0.125 mg QD of the comparator is not expected to have a significant pharmacological effect (Table 1) but the same dose of AQ001S (0.125 mg) is expected to have detectable pharmacological activity. Dose/efficacy response curves of ICS are indeed sigmoidal [39,40] and, based on the results of nonclinical experiments, Aquilon expects AQ001S 0.125 mg/ml ( ) to show higher efficacy in Figure 2, than the comparator used at the same dose.

Although AQ001S has the potential to be administered with any nebulizer device, this parameter will not be investigated in the present clinical trial. The use of a conventional jet nebulizer enables a proper comparison of the solution and the suspension comparator administration.
This prospective clinical trial will be conducted in accordance with the protocol and with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice (GCP) and the applicable regulatory requirement(s), including the Belgian regulation (country where the clinical trial will be conducted).

## 6.3. Benefit-Risk Statement

### 6.3.1. Benefits and Risks Related to the Investigational Medicinal Products

In participating to the BOREAS clinical trial, the subjects will contribute to the process of developing new therapies in an area of unmet need. The subjects will be closely monitored and followed during their participation and this might help them to better understand and/or manage their disease.
The subjects selected for this trial will be adult patients who have persistent, mild asthma for at least 3 months, using as-needed reliever medication only (SABA-containing medication) and who are naïve to ICS for at least 60 days at clinical trial entry. Subjects will be objectively selected, i.e. according to functional tests to evaluate the severity of their asthma. The treatment they will receive (at least AQ001S 0.125 mg/ml) aims at stabilizing their asthma, and therefore at avoiding asthma exacerbations (maintenance treatment).

ICS as maintenance treatment of asthma are routinely used at significantly higher doses and for longer duration periods than the dose and the treatment periods planned in the BOREAS trial (2 periods of 29 [+2] days). The ICS dose chosen for the clinical trial is expected to lead to less adverse reactions (ARs).

The benefits of AQ001S *versus* the currently marketed budesonide liquid formulations, that may rely on the use of the HP-Betadex excipient, are described in Section 6.1, i.e. the possibility to use AQ001S with any nebulizer device and a decrease of patient exposure to ICS with a reduction of ICS-related AEs. The use of HP-Betadex in inhaled drugs is new. The nonclinical, clinical and toxicology studies conducted with inhaled HP-Betadex and AQ001S do not point out any safety concerns for the intended clinical dose strengths (also further described and summarized in AQ001S' IB).
However, safety is an endpoint of the present clinical trial and the low dose chosen is a risk minimization measure.

The potential benefits and risks of AQ001S are further described and summarized in AQ001S' IB The potential benefits and risks of Budesonide inhalation suspension (comparator) are reviewed in its summary of product characteristics (SmPC)

The dose strengths of both IMPs that will be administered during this clinical trial are low and chosen on purpose to demonstrate AQ001S' superiority *versus* the comparator. Even if 0.125 mg QD budesonide inhalation suspension, i.e. the comparator, is not efficient, this should have no negative impact on the asthma control of the mild asthmatic subjects that will be selected to participate in this clinical trial. Moreover, the clinical trial allows the subjects to continue their usual treatment with as-needed reliever medication (SABA only).

### 6.3.2. Benefits and Risks Related to the Trial Procedures

Methacholine (MCh) challenge testing will be performed in this clinical trial to determine the primary efficacy parameter PC20 (concentration of MCh provoking a fall of 20% of the forced expiratory volume in 1 second [$FEV_1$]), with the use of MCh as a provocative agent (Section 9.2.2.1).
Methacholine is a classical provocative agent used in asthma diagnosis and the related safety measures are well described [41] and part of the routine procedures of the trial site. The trial subject will always be overseen by qualified site staff, under the responsibility of the Principal Investigator. After each challenge, spirometry assessments are scheduled (Section 9.2.1.7) to measure $FEV_1$ and, along with the presence of asthma-related clinical symptoms (Section 9.2.1.6), to determine the need to administer as-needed reliever medication (SABA) to the subject (if $FEV_1 < 70\%$; Section 9.2.1.8). In that case and at any time during the clinical trial, as-needed reliever medications will be available at trial site.
The potential benefits and risks of the MCh (Provocholine®) used in this clinical trial are summarized in its technical information sheet.
For the PK analyses, 16 blood samples will be taken at Visits 1 and 3, and 7 blood samples at Visits 2 and 4. The total blood volumes, combined with those necessary for the clinical laboratory tests (Section 9.2.1.2) and blood eosinophil count (Section 9.2.2.5), are mentioned in Section 9.2.1.2.2, i.e.:

- a maximum of 72.5 ml of blood taken per visit.
- an estimated total maximum blood volume needed for a subject during the trial (when completing the trial up until the last visit) of 260.5 ml, over 77 to 85 days.

These blood volumes are below the maximum allowable blood draws as recommended by:

- the Duke University Health System - Human Research Protection Program [42], i.e. for an adult, not more than 5 ml/kg in any one 24-hour period, and 7 ml/kg in any 8-week period, and
- the Dana Farber Harvard Cancer Center [43], i.e. not more than 275 ml over a 28-day period.

The IMPs will be administered by nebulization with a Pari Boy Classic compressor combined with Pari LC Sprint nebulizer. The warnings and precautions of use related to these devices are described in their instructions for use (IFU) (Appendix 1 and Appendix 2).

### 6.3.3. Benefit-Risk Conclusion

The mild asthmatic adult subjects entering this clinical trial will receive either an innovative IMP (under investigation: AQ001S inhalation solution) or a well-established and already marketed IMP (comparator: Budesonide inhalation suspension), both at a low dose of 0.125 mg budesonide QD, which are expected to induce less ARs than those expected for recommended doses of comparator. If AQ001S 0.125 mg/ml is shown to be superior to the comparator, the subject's participation will contribute to the process of developing new therapies in an area of unmet need. The subjects could then benefit from those therapies right at the commercialization stage if successful, as it will open new therapeutic perspectives in asthma management (Section 6.1).

Participating subjects should be ICS-naïve for the last 60 days, meaning that their asthma is sufficiently controlled with as-needed reliever medication only. Therefore, their condition could not deteriorate if one IMP treatment is not efficient.

The benefit/risk ratio of AQ001S 0.125 mg/ml will be evaluated continuously by the Sponsor's Pharmacovigilance Manager who will liaise with the Principal Investigator. Decision on clinical trial or subject discontinuation will be taken after proper common evaluation of AQ001S 0.125 mg/ml benefit/risk ratio by the Sponsor's Pharmacovigilance Manager and the Investigator.

The risks associated with the trial assessments, in particular for the MCh challenge testing and the PK sampling, are known, limited and mitigated (Section 6.3.2).

Finally, the subjects will be closely and objectively selected, i.e. according to functional tests performed to evaluate their asthma severity, and they will be closely monitored during their whole participation in the clinical trial, including during the home-visits by study nurses.

# 7. CLINICAL TRIAL PURPOSE AND OBJECTIVES

## 7.1. Purpose of the Clinical Trial

The goal of this clinical trial is to establish the POC of AQ001S 0.125 mg/ml by assessing and comparing its efficacy, safety and PK to those of the comparator.

## 7.2. Primary Objectives

The primary objectives of the clinical trial are:

- To compare the efficacy, i.e. the bronchoprotection, of AQ001S 0.125 mg/ml with the comparator.
- To assess the safety of AQ001S 0.125 mg/ml.

## 7.3. Secondary Objectives

The secondary objectives are:

3. To compare the PK of AQ001S 0.125 mg/ml with the comparator
4. To compare the secondary efficacy/PD of AQ001S 0.125 mg/ml with the comparator.

# 8. INVESTIGATIONAL PLAN

## 8.1. Primary and Secondary Parameters

### 8.1.1. Primary Efficacy Parameter

Bronchoprotection will be assessed by determining PC20, as determined by MCh challenge test.

### 8.1.2. Safety Parameters

The safety will be evaluated collecting the following information:

- Adverse events (AEs) and serious adverse events (SAEs), including asthma exacerbations
- General tolerability: vital signs, 12-lead electrocardiogram (ECG), physical examination
- Laboratory parameters: hematology, biochemistry and urinalysis
- Local tolerability:
  - Increased bronchial irritability
  - Paradoxical bronchospasm
  - Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection)
- Forced expiratory volume in 1 second ($FEV_1$) and forced vital capacity (FVC), measured by spirometry
- Assessment of hypothalamic pituitary adrenocortical (HPA) axis function: urinary cortisol/creatinine ratio in first waking urine.

### 8.1.3. Pharmacokinetic Parameters

The PK profile of budesonide in plasma will be evaluated for each treatment period at:

- Start of treatment period:
  - single dose 24-hour PK parameters at the time of the first scheduled IMP administration (Section 9.2.3.1 and 9.2.3.4).
- End of treatment period:
  - abridged PK at the time of the last scheduled IMP administration (Section 9.2.3.1 and 9.2.3.4).

### 8.1.4. Secondary Pharmacodynamic/Efficacy Parameters

The secondary efficacy/PD will be evaluated using the following parameters:

- Symptom scores will be recorded using day- and night-time asthma symptom scoring and validated asthma-specific Asthma Control Questionnaire (ACQ)-5 and Asthma Control Test (ACT) questionnaires (included in trial subject diary).
- Use of as-needed reliever medication (reported in trial subject diary).
- Airway inflammation PD biomarkers: fractional concentration of exhaled nitric oxide (FeNO) and blood eosinophil count.
- $FEV_1$ and forced inspiratory vital capacity (FIVC), measured by spirometry.

## 8.2. Overall Clinical trial Design and Plan

This is a prospective, active-controlled, randomized, open label, single-center, multiple dose, two-period crossover clinical trial in adults with mild asthma. This clinical trial is conducted in a single trial site in Belgium. Twenty-four (24) ICS-naïve for minimum 60 days, mild asthmatic adults (18 to 65 years old) will be recruited. Considering potential dropouts, a total of 20 subjects are anticipated to complete the clinical trial.

Eligible subjects, i.e. fulfilling the criteria defined in Section 8.4 and having given their informed consent, will be randomized at Visit 1 in a 1 to 1 ratio to receive, for a first treatment period, one of the following IMPs:

- AQ001S 0.125 mg/ml or
- The comparator (0.125 mg/ml).

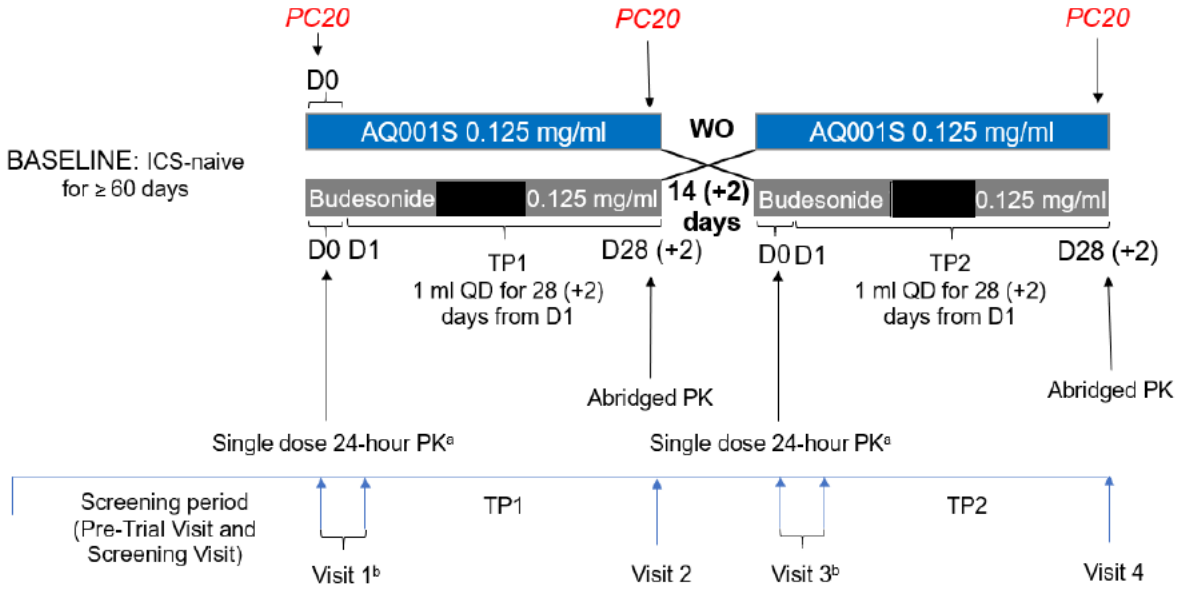
All subjects will receive the randomly allocated IMP for 29 (+2) days. After a 14 (+2) days washout period, the subjects will receive the other IMP for a second treatment period of 29 (+2) days (crossover design).

For each subject, the clinical trial will last 77 to 85 days from Screening Visit, including two treatment periods of 29 (+2) days and one washout period of 14 (+2) days, between the treatment periods.

In case the subject is taking or has taken ICS within the last 60 days at the time of the Pre-Trial Visit (the visit before or combined to the Screening Visit), the total duration might be a maximum of 147 days from Pre-Trial Visit.
The inclusion period is anticipated to last at least 3 months. The clinical trial is estimated to last 6 months from first-subject-in to last-subject-out.

Clinical trial duration and general procedures are presented schematically in Figure 3. The flow chart of assessments is summarized in Section 3 and detailed per clinical trial visit in Section 9.1.

**Figure 3 - BOREAS clinical trial design**




D = treatment day; ICS = inhaled corticosteroid; MCh = methacholine (chloride); PC20 = concentration of MCh provoking an $FEV_1$ fall of 20%; PK = pharmacokinetics; TP = treatment period; QD = once daily; WO = washout
[a] 2 ml IMP (total of 0.250 mg budesonide) administered at D0 for the single dose 24-hour PK.
[b] Visits 1 and 3 include the D0 and D1 administrations and last approximately 30 hours.

## 8.3. Discussion on Clinical Trial Design and Choice of Control Group(s)

### 8.3.1. Justification of Clinical Trial Design

The clinical trial has been designed to allow comparing the efficacy and the PK of AQ001S 0.125 mg/ml *versus* comparator (0.125 mg/ml).

In addition, the safety of AQ001S 0.125 mg/ml (first administration in humans of this formulation of the well-known budesonide) *versus* comparator will be assessed.

In this clinical trial, mild asthmatic subjects have been preferred to healthy volunteers to assess the safety of AQ001S 0.125 mg/ml as it allows the collection, in the same subjects, of relevant efficacy, PD and PK data, which would not have been possible in healthy volunteers.

In order to distinguish the effects of the treatments that will be administered in the BOREAS clinical trial, the subjects will be ICS-naïve for at least 60 days before the Screening Visit. Therefore, in case the subject is taking or has taken ICS within the last 60 days at the time of the Pre-Trial Visit, the subject will have to follow a washout period of 60 (+2) days before the Screening Visit.

Based on the available nonclinical data, the primary hypothesis is that AQ001S 0.125 mg/ml has a higher efficacy than the comparator (Section 6.2 and Figure 2). This clinical trial intends to compare several pulmonary efficacy and PD parameters. Due to interindividual variability of the efficacy and PD parameters measured, a crossover design has been chosen. This will expose fewer subjects to IMPs than a parallel design, with a higher chance to show a difference between IMPs.

The budesonide PK parameters will be compared between AQ001S 0.125 mg/ml and the comparator. The PK parameters are not anticipated to correlate with the efficacy or PD as most of the budesonide therapeutic action is due to the local pulmonary effects after inhalation. The PK analysis will however determine if and how .

Budesonide shows its anti-inflammatory effects after approximately 10 days [38]. Therefore, the treatment duration was set to a minimum of 28 days at the dose strength of 0.125 mg/ml budesonide once daily (0.125 mg/day) per treatment period as this is long enough to demonstrate potential efficacy of both IMPs.

A washout period of 14 (+2) days between the treatment periods has been selected to avoid a potential carry-over effect [44].

### 8.3.2. Choice of Control Group(s)

Following the crossover design, each subject will be his/her own control avoiding the problem of interindividual variability related to a parallel design.

The comparator, Budesonide , has been chosen as it is a generic of the original Pulmicort Respules®, i.e. its bioequivalence to the original formulation is well established. The strength of 0.125 mg/ml has been selected to administer the same dose of both AQ001S 0.125 mg/ml and the comparator and to show the superiority of AQ001S over the comparator at this strength, as explained in Section 6.2 and Figure 2.

### 8.3.3. Choice of Clinical Trial Parameters

#### 8.3.3.1. *Primary Efficacy Parameter*

**Methacholine challenge test and PC20**

ICS require a sensitive lung function parameter to demonstrate their effect as anti-inflammatory maintenance treatment in asthma.

$FEV_1$ is a classical lung function parameter [45] with a good specificity for asthma diagnosis but its intraindividual variation (between visits) gives it a poor sensitivity, e.g. compared with bronchial challenge testing [46]. Therefore, its use as primary efficacy endpoint may require a high number of enrolled subjects in order to demonstrate the POC for AQ001S, i.e. its superiority *versus* the comparator. This would lead to a sample size that is not desirable in a FIH clinical trial.

Methacholine (MCh) challenge test is one of the most reported and used methods for assessing airway responsiveness [45], as described in the guideline of the American Thoracic Society [41]. During this test, PC20 (concentration of MCh provoking an $FEV_1$ fall of 20%) is the classical measured parameter. Moreover, as a bronchial challenge test, its sensitivity is superior to the one of the classical lung function parameter $FEV_1$.

The sample size calculated for the use of PC20 (Section 11.1) allows to include a reduced number of subjects in this FIH POC clinical trial.

#### 8.3.3.2. *Safety Parameters*

The following specific safety parameters have been added to the classical ones (recording and reporting of AEs, SAEs, vital signs, physical examination, laboratory testing, ECG):

- Specific AEs: asthma exacerbations [45]
- Local tolerability:
    a. Increased bronchial irritability [47]
    b. Paradoxical bronchospasm [47]
    c. Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection associated with ICS use [45])
- $FEV_1$ and FVC, measured by spirometry to assess airflow limitation [45]
- HPA axis function, assessed through urinary cortisol/creatinine ratio measurement in first waking urine.

Spirometry is a classical clinical test of the pulmonary function. $FEV_1$ and FVC are listed as safety parameters and aim at detecting any degradation of the pulmonary function.

Urinary cortisol/creatinine ratio in first waking urine has been chosen over 24-hour plasma cortisol [45] to evaluate the commonly described effects of ICS on the HPA axis, as recommended in the literature, especially when conducting clinical trials [48,49]. Given the limited number of subjects that will participate in this clinical trial, it will be an exploratory parameter. The timing of first waking urine sampling in the morning is more flexible than the strict timing required for blood sampling collection to measure the 'single morning plasma cortisol' (urine: between 6 and 9 AM; *versus* blood: between 7.30 and 8.30 AM) [49]. Moreover, it will be easier for the subject to collect the first waking urine in the morning before each clinical trial visit, rather than the standard 24-hour urine collection, which can be cumbersome, especially when combined with a professional life.
Population reference values for the urinary cortisol/creatinine ratio in first waking urine for urine collection between 6 and 9 AM have been established by the local laboratory in charge prior to the clinical trial (Section 1).
Of note, blood cortisol will also be measured in the set of hematology and biochemistry analysis. However, the timing of sampling might be inconstant between visits due to the number of procedures to be conducted at each visit. Hence, in contrast to the urinary cortisol/creatinine ratio in first waking urine, single morning plasma cortisol values will be considered as indicative and not included in the specific statistical analysis.

#### 8.3.3.3. *Pharmacokinetic Parameters*

The PK analysis will determine ,for each treatment period:

- through a single dose 24-hour PK at the first IMP administration
- through an abridged PK at the time of the last IMP administration.

To establish the single dose 24-hour PK profiles of both AQ001S 0.125 mg/ml and the comparator on the first day of administration of each treatment period, a dose of 0.250 mg budesonide of each IMP will be administered by nebulization. This dose has been chosen in order to ensure that detectable plasma concentrations of budesonide can be reached over sufficient time to completely assess budesonide elimination phase (Section 9.2.3.1).

#### 8.3.3.4. *Secondary Efficacy/Pharmacodynamic Parameters and Techniques*

Besides the primary efficacy parameter, classical asthma efficacy/PD parameters will be evaluated in the BOREAS clinical trial:

- Symptom scores will be recorded through the use of day- and night-time asthma symptom scoring and validated asthma-specific ACQ-5 and ACT questionnaires (included in trial subject diary) [45].
- Use of as-needed reliever medication (reported in trial subject diary) [45].
- Airway inflammation PD biomarkers: FeNO [45] and blood eosinophil count.
- $FEV_1$ and FIVC, measured by spirometry [45].

The blood eosinophil count (Section 9.2.2.5) is a surrogate biomarker of eosinophilic airway inflammation, a common feature of specific asthma phenotypes. Although less sensitive, it has been preferred to the sputum eosinophil count [45] as it has been reported to be less variable and less labor intensive for the subject than the sputum collection procedure [50].

## 8.4. Clinical Trial Population

### 8.4.1. Population Base

Adults with mild asthma (18 to 65 years old), who are ICS-naïve for minimum 60 days at Screening Visit.

### 8.4.2. Inclusion Criteria

Potential trial subjects may be entered in the clinical trial if they meet all of the following criteria:

1. Reliable subjects who are willing to be available for the duration of the clinical trial and willing to comply with clinical trial procedures.
2. Subjects who have the ability to understand the requirements of the clinical trial.
3. Subjects who have given written informed consent.
4. Subjects aged between 18 and 65 years, inclusive.
5. Body mass index between 18.5 and 29 $kg/m^2$.
6. Documented clinical diagnosis of stable, persistent, asthma for at least 3 months, i.e.
    - for whom $FEV_1 \geq 70\%$ of predicted, and
    - treated with as-needed reliever medication (SABA-containing medication) only.
7. Subjects who are ICS-naïve for minimum 60 days at Screening Visit.
8. Positive MCh challenge test (PC20 < 8 mg/ml or PD20 < 0.2 mg) in the last year.
9. Post-bronchodilator $FEV_1$ at least 80% of the predicted documented in the last year.
10. Clinical laboratory test results, 12-lead ECG, blood pressure and heart rate (supine) within normal reference range or judged to be not clinically significant by the Investigator.
11. Female subjects of childbearing potential should have a negative pregnancy test at Screening Visit.
12. Female subjects of childbearing potential using a highly effective method of contraception (i.e., pregnancy rate of < 1% per year) on a stable regimen, for at least 28 days, and pursuing this contraception during the trial and for 28 days after the last administration of the IMP. The highly effective methods of contraception must be one of the following: combined estrogen and progestogen hormonal contraception with inhibition of ovulation, progestogen-only hormonal contraception associated with inhibition of ovulation, intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner, or agreement on continuous abstinence from heterosexual intercourse.

### 8.4.3. Exclusion Criteria

Potential trial subjects will not be entered into the clinical trial if any of the following apply:

1. Current smokers or recent (< 8 weeks) ex-smokers or ex-smokers if > 10 pack-years.
2. Pregnant or breastfeeding female subjects.
3. Inability to carry out pulmonary function testing.
4. $FEV_1$ < 70%.
5. History of near-fatal asthma and/or intensive care unit admission for asthma symptoms.
6. Exacerbations of asthma requiring oral steroids, hospitalization or change in asthma treatment in the previous three months.
7. Evidence of symptomatic chronic or acute respiratory infection in the previous 8 weeks.
8. Diagnosis of chronic obstructive pulmonary disease (COPD) or bronchiectasis.
9. Pulmonary malformations, tuberculosis, cystic fibrosis.
10. History of hypersensitivity or existing contraindication to budesonide or any other IMP ingredients.
11. Untreated oral candidiasis.
12. Immunosuppressive treatment, including systemic corticosteroids (e.g., oral, parenteral, ocular, nasal), within 28 days before Screening Visit.
13. Use of ICS within 60 days before Screening Visit.
14. Use of anti-leukotrienes, immunoglobulins, beta-blockers, digitalis, amiodarone, antifungals, macrolides, antidepressants, monoamine oxidase inhibitors, antiretroviral drugs, cholinesterase inhibitors, histamine, theophylline, non-steroidal anti-inflammatory drugs, anticholinergic drugs, neuroleptics, curariform drugs, antihistaminic (anti-H1) drugs, calcium channel blockers, LABAs, mast cell stabilizers (e.g. natrium cromoglycate).
15. History of alcohol or drug abuse.
16. Unstable or life-threatening cardiac disease: subjects with any of the following would be excluded:
    - Myocardial infarction or unstable angina in the 6 months before Screening Visit,
    - Unstable or life-threatening cardiac arrythmia requiring intervention in the 3 months prior to Screening Visit,
    - New York Heart Association class IV Heart failure.
17. History or presence of prolonged QT interval (> 470 ms), or any other clinically significant ECG abnormalities as judged by the Investigator based on 12-lead ECG recordings at Screening Visit.
18. Diabetes mellitus.
19. Neuropsychiatric diseases.
20. Clinically relevant laboratory abnormalities at Screening Visit.
21. Blood or plasma donation within 30 days prior to Screening Visit.
22. History or presence of malignancy of any system organ class (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years prior to Screening Visit, regardless of whether there is evidence of local recurrence or metastases.
23. Any surgical or medical condition which might significantly alter the absorption, distribution, metabolism, or excretion of drugs, or which may jeopardize the subject in case of participation in the clinical trial.
24. History or presence of any other clinically relevant disease of any major system organ class (e.g. cardiovascular, pulmonary, renal, hepatic, gastrointestinal, reproductive, endocrinological, neurological, psychiatric or orthopedic disease) as judged by the Investigator.
25. Human immunodeficiency virus (HIV) and SARS-CoV-2 infections.
26. Subjects who participated in an investigational trial within the 12 weeks prior to the start of the trial.

### 8.4.4. Screening Failures

The final subject's eligibility will be assessed at Visit 1. Assessments linked to inclusion and exclusion criteria at Screening Visit and the confirmatory results obtained at Visit 1 will be used to determine the eligibility of each subject for randomization at Visit 1, including the review of prior medications (Section 8.8.1). Subjects who fail to meet the eligibility criteria will be considered as screening failures and not as subject's withdrawal. Screening failures will be replaced automatically.
A list of screening failures will be maintained by the Investigator, mentioning the reason for exclusion/non-inclusion. At the end of the clinical trial, the Investigator will communicate the number of subjects screened and excluded with a breakdown of the reasons for excluding subjects during screening.

### 8.4.5. Subject Identification

Subject trial identification (ID) numbers will be assigned at Pre-Trial Visit (Screening ID), with definite allocation at Visit 1 (Randomization ID).

Screening ID will consist of 8 digits according to the following numbering structure:

S-BE-01-XXX

Where:
S = screening
BE = country code (Belgium in this trial)
01 = trial site number (only one in this trial)
XXX = subject's allocated ID at screening, i.e. 3 digits beginning with 001 and consecutive.

Randomization ID will consist of 7 digits according to the following numbering structure:

BE-01-YYY

Where:
BE = country code (Belgium in this trial)
01 = trial site number (only one in this trial)
YYY = subject's allocated ID at randomization, i.e. 3 digits beginning with 001 and consecutive.

Randomization IDs will then be used throughout the trial.

The Investigator or designee will enter both the Screening ID and Randomization ID in both the electronic case report form (eCRF) and the confidential subject identification log.

## 8.5. Investigational Medicinal Products

### 8.5.1. Treatments

The subjects will receive their assigned intervention according to the sequence determined by the randomization procedure (Sections 8.9 and 11.2). The interventions are:

- At D0 of each treatment period, i.e. for the single dose 24-hour PK determination:
  - AQ001S treatment = one single dose of 2 ml of AQ001S 0.125 mg/ml (total dose of 0.250 mg budesonide) by nebulization.
  - Comparator treatment = one single dose of 2 ml of Budesonide 0.125 mg/ml (total dose of 0.250 mg budesonide) by nebulization.

- Then from D1 to the end of each treatment period:
  - AQ001S treatment = single dose of 1 ml of AQ001S 0.125 mg/ml (total dose of 0.125 mg budesonide) QD for 28 (+2) days by nebulization.
  - Comparator treatment = single dose of 1 ml of Budesonide 0.125 mg/ml (total dose of 0.125 mg budesonide) QD for 28 (+2) days by nebulization.

### 8.5.2. Name and Description of Investigational Medicinal Products

One single batch of AQ001S 0.125 mg/ml and one single batch of the comparator will be used for the clinical trial.

AQ001S 0.125 mg/ml is a sterile transparent solution containing 0.125 mg/ml budesonide and mg/ml of the novel excipient HP-Betadex (see AQ001S' IB). AQ001S 0.125 mg/ml is manufactured following Good Manufacturing Practices by the manufacturer mentioned in Section 1.

Budesonide 0.125 mg/ml inhalation suspension will be used as the comparator treatment (see SmPC). It is a commercial product, approved and marketed in Europe.

### 8.5.3. Route of Administration

Both IMPs are orally inhaled products and will be administered by nebulization.

### 8.5.4. Packaging and Labelling

Packaging and labelling of the IMPs will comply with Annex 13 of the European Union Guidelines to Good Manufacturing Practice and with Belgian requirements applicable to IMPs and will be performed by the subcontractor mentioned in Section 1.

Both IMPs will be supplied as single-dose low density polyethylene 2-ml ampoules, packed in aluminum pouches (primary packaging) and cardboard boxes (secondary packaging) as follows:

| **AQ001S 0.125 mg/ml** | **Budesonide 0.125 mg/ml** |
|---|---|
| Pouches of 10 ampoules | Pouches of 5 ampoules |
| Boxes of 40 ampoules, i.e. 4 pouches | Boxes of 40 ampoules, i.e. 8 pouches |

The labels affixed to the primary and secondary packaging of both IMPs will contain the following information:

- Randomization ID
- Trial code (BOREAS)
- Treatment Group (e.g. A or B, see Section 8.9)
- Treatment Period (e.g. 1 or 2)
- Investigator:
- Sponsor: Aquilon Pharmaceuticals (Liège, BE)
- FOR CLINICAL TRIAL USE ONLY - Keep out of reach of children
- Administration via nebulization
- Do not store above 25°C; do not freeze
- Store the unopened ampoules in the aluminum pouch to protect them from light, in an upright position. Use within of opening the pouch.
- Batch Nr
- According to the IMP:

| **AQ001S 0.125 mg/ml** | **Budesonide 0.125 mg/ml** |
|---|---|

| | |
|---|---|
| Budesonide solution for inhalation 0.125 mg/ml (number of units) | Budesonide 0.125 mg/ml (number of units) |
| Retest date | Expiry date |

## 8.5.5. Conditions for Storage and Use

Both IMPs should be stored as follows:

- Between
- Not above 25°C at subject's home.

Both IMPs should not be frozen.
Any opened ampoule should be used immediately, and the unused portion discarded as described in Section 8.12.
Store the unopened ampoules in the aluminum pouch to protect them from light, in an upright position. Use within of opening the pouch when stored between
The Investigator's authorized site staff will ensure that the IMPs are stored in appropriate conditions with restricted access and in compliance with national regulations.
On D1 of Visit 1 and Visit 3, the subjects will receive their allocated IMP box for the corresponding treatment period. From D2, at the subject's home, the study nurses will every day, until D28 (+2), take 1 ampoule out of the allocated box for subject administration as described in Section 8.5.7.

## 8.5.6. Dosage and Dosage Regimen

At the start of each treatment period, i.e. on D0, a volume of 2 ml AQ001S or comparator (corresponding to a dose of 0.250 mg budesonide) will be administered for the single dose 24-hour PK at the trial site.
On D1 of each treatment period, a volume of 1 ml AQ001S or comparator (corresponding to a dose of 0.125 mg budesonide) will be administered at the trial site.
From D2 to the last day of home administration of each treatment period, a volume of 1 ml AQ001S or comparator (corresponding to a dose of 0.125 mg budesonide) will be administered QD in the morning at home by a study nurse.
On D28 (+2) of each treatment period, a volume of 1 ml AQ001S or comparator (corresponding to a dose of 0.125 mg budesonide) will be administered for the abridged PK at the trial site.

There will be an interval period of 24 ± 2 hours between 2 doses, except between D1 (at trial site) and D2 (in the morning at home) of each treatment period, where there will be approximately an interval period of 24 (- 4) hours between the administrations.

## 8.5.7. Preparation and Method of Administration

Both IMPs will be administered with a standard jet nebulizer (Pari Boy Classic compressor with Pari LC Sprint nebulizer; see respective IFU in Appendix 1 and Appendix 2).
At D0, for the first administration for single dose 24-hour PK determination, the Investigator or the study nurse will transfer the whole content of the 2-ml ampoule containing either AQ001S or the comparator (both at the strength of 0.125 mg/ml, i.e. 0.250 mg/2 ml) to the nebulizer. This leads to a total nebulization volume of 2 ml containing a total dose of 0.250 mg budesonide to be inhaled.
During the 2 treatment periods, from D1 to D28 (+2), the Investigator or the study nurse will transfer 1 ml of the 2-ml ampoule containing either AQ001S or the comparator (both at the strength of 0.125 mg/ml) into the nebulizer cup and add 1 ml of sterile normal saline. This leads to a total nebulization volume of 2 ml containing a total dose of 0.125 mg budesonide to be inhaled.
The subject will inhale the prepared IMP through the provided mouthpiece until the total medication volume has been nebulized, as described in the IFU of the Pari LC Sprint nebulizer.
Subjects will be instructed to rinse their mouth and spit out the rinse fluid after the nebulization procedure.

The nebulization for daily dose administration is not dependent on food intake as the IMPs are administered by inhalation.

## 8.6. Auxiliary Medicinal Product Used in the Clinical Trial

### 8.6.1. Name and Description of Auxiliary Medicinal Product

The MCh challenges (Section 9.2.2.1) will be performed using the authorized auxiliary medicinal product (AxMP) Provocholine® as provocative agent. It is presented as a powder and will be resuspended in sterile normal saline at the desired concentration by the clinical staff at trial site. MCh will be administered to the subjects only by the Investigator and/or site staff, according to the guidelines of the American Thoracic Society [41]. This AxMP is not subject-specific.

Provocholine® will be shipped from the supplier directly to the trial site after site initiation visit and stored according to recommended storage conditions. It will remain at the trial site and will only be handled by authorized site staff.

### 8.6.2. Labelling of Auxiliary Medicinal Product

Labelling of the AxMP will comply with Chapter X and Annex VI of Regulation (EU) No 536/2014 and will be performed by the subcontractor mentioned in Section 1.

The labels affixed to the primary and secondary packaging of the AxMP will contain the following information, taking into account that the AxMP is not subject-specific, that it will remain at the trial site and that it will only be handled by authorized site staff:

- Trial code (BOREAS)
- Investigator:
- Sponsor: Aquilon Pharmaceuticals (Liège, BE)
- FOR CLINICAL TRIAL USE ONLY

### 8.6.3. Traceability and Destruction of Auxiliary Medicinal Product

The batch number of the AxMP that will be used for each MCh challenge per subject will be recorded in the eCRF.
At the end of the clinical trial, unused and partially used AxMPs must be returned to the Sponsor or Sponsor's designated subcontractor for destruction.

## 8.7. Medical Device Used in the Clinical Trial

The standard jet nebulizer that will be used to administer the IMPs is the Pari LC Sprint nebulizer, combined with a Pari Boy Classic compressor. They are CE-marked and will be used in accordance with their respective IFUs provided in Appendix 1 and Appendix 2.

## 8.8. Prior Treatments and Concomitant Medications

### 8.8.1. Prior and Concomitant Medication

Details on prior medications and on any concomitant medications taken during the clinical trial will be recorded according to Section 9.2.4.4, i.e. from 3 months prior to the Screening Visit.

Any medications taken before the first IMP administration at Visit 1 will be recorded by the Investigator as prior medications. Any medications taken from the first IMP administration at Visit 1 will be recorded by the Investigator as concomitant medications (Section 9.2.4.4).

The Investigator will evaluate the medication information collected in view of the inclusion and exclusion criteria to ensure that the list of medications is not indicative of a pathology listed in the exclusion criteria. In case the medication history demonstrates that one or more of the inclusion/exclusion criteria are not met, the subject will be withdrawn before randomization. This subject will be replaced and considered as a screening failure (Section 8.4.4).

### 8.8.2. Forbidden and As-Needed Reliever Medication

Any type of asthma-related medication is forbidden during the clinical trial except the use of a SABA as as-needed reliever medication in case of asthma crisis necessitating treatment. In case the subject takes another asthma-related medication than the authorized as-needed medication (SABA only), the Investigator will document it and consider subject withdrawal after discussion with Sponsor's medical monitor (Section 10.5.1).

Other forbidden concomitant medications are:

- Immunosuppressive treatment, including systemic corticosteroids (e.g., oral, parenteral, ocular, nasal).
- ICS
- Anti-leukotrienes, immunoglobulins, beta-blockers, digitalis, amiodarone, antifungals, macrolides, antidepressants, monoamine oxidase inhibitors, antiretroviral drugs, cholinesterase inhibitors, histamine, theophylline, non-steroidal anti-inflammatory drugs, anticholinergic drugs, neuroleptics, curariform drugs, antihistaminic (anti-H1) drugs, calcium channel blockers, long-acting beta2-antagonists, mast cell stabilizers (e.g. natrium cromoglycate).

The Investigator and the subject's treating physician should however always consider the interest of the subject as the priority. Therefore, in case any forbidden concomitant medication is needed by the subject, they will consider the subject's interest rather than the clinical trial continuation.

Any concomitant medications will be recorded according to Section 9.2.4.4 and the subjects will record the use of as-needed reliever medications (SABA only) in their diaries (Section 9.2.2.2).

## 8.9. Assignment of Subjects to Treatment Groups

Eligible subjects, i.e. fulfilling the criteria defined in Section 8.4 and having given their informed consent, will be randomized in a 1 to 1 ratio to one of the following treatment sequences at Visit 1 (crossover design):

| Treatment sequence A | Treatment sequence B |
|---|---|
| Treatment period 1: AQ001S 0.125 mg/ml for 29 (+2) days | Treatment period 1: comparator 0.125 mg/ml for 29 (+2) days |
| Washout for 14 (+2) days | Washout for 14 (+2) days |
| Treatment period 2: comparator 0.125 mg/ml for 29 (+2) days | Treatment period 2: AQ001S 0.125 mg/ml for 29 (+2) days |

The randomization of the subjects will be performed according to Section 11.2.
The Investigator will inform the monitor of new randomized subjects.

## 8.10. Blinding and Emergency Cards

This is an open label clinical trial.
The subject emergency card will mention his/her participation in the clinical trial as well as emergency phone numbers of the Principal Investigator.

## 8.11. Treatment Compliance

AQ001S 0.125 mg/ml and comparator will be administered to the subjects both at the trial site (PK, first dose and last dose of each treatment) and at the subject's home where the IMP will be prepared by the study nurse (for the whole treatment period except first and last dose). For each administration, trial staff and study nurses will fill in the nebulizer device with either of the IMPs as described in Section 8.5.7 and will initiate the inhalation for the subject. For that purpose, the study nurses will receive training on IMP administration and handling prior to any subject visits. They will document the treatment procedures in a log, including IMP identification (Randomization ID, treatment group and treatment period), date and time of administration, allowing the monitoring of treatment compliance.

At the first home visit, the study nurse will bring the nebulizer to the subject's home. The nebulizer will then stay at subject's home until the last home IMP administration of the clinical trial. After this last home administration, the nebulizer will be brought back to the trial site.

## 8.12. Drug Dispensing and Accountability

Both AQ001S 0.125 mg/ml and comparator batches will be shipped to the trial site after site initiation visit and stored according to recommended storage conditions.

The IMPs will be dispensed to the subjects by the Investigator or authorized trial staff at the time points/visits indicated in the flowchart in Section 3.

Any IMPs provided to the site will be accounted for. This includes IMPs received at the site, IMPs dispensed to the subjects, and unused and partially used IMPs returned by the study nurse.

A drug inventory and dispensing log will be kept current by the Investigator or authorized trial staff, detailing quantities of IMPs received (including information about time and date), as well as quantities dispensed to each subject (including information about time and date) and the remaining quantity. The drug inventory and dispensing log will be available to the monitor to verify drug accountability during the clinical trial.

At the subject's home, the study nurse will collect the used and partially used IMPs in a disposal bin and will return them to the trial site as soon as feasible. At Visits 2 and 4, i.e. after the last home administration of each treatment period, the subjects will bring back the unused IMPs.
The drug inventory and dispensing log will be updated accordingly by the Investigator and authorized trial staff to ensure the drug accountability.

For every subject, the drug accountability of his/her first treatment period should be closed during visit 2 at trial site before starting his/her second treatment period, so to avoid that first treatment doses are still in circulation when starting the second treatment period.

At the end of the clinical trial, unused and partially used IMPs must be returned to the Sponsor or Sponsor's designated subcontractor for destruction.

Destruction can be initiated only after accountability has been verified and fully reconciled by the monitor and after the Sponsor has granted written approval of destruction.

# 9. CLINICAL TRIAL CONDUCT

The flow chart of assessments planned for each trial visit is given in Section 3. The clinical trial will include:

- one Pre-Trial Visit,
- one Screening Visit,
- one Randomization and Baseline Visit (Visit 1), with the start of the first treatment period

- three intermediate visits (Visits 2 to 4), with a washout period of 14 (+2) days between Visit 2 and Visit 3.
- The Visit 4 is also the End-of-Trial visit.

Visit 1 and Visit 2 correspond to the beginning and the end of the first treatment period (TP1), respectively.
Visit 3 and Visit 4 correspond to the beginning and the end of the second treatment period (TP2), respectively.

## 9.1. Procedures by Visit

The procedures described in this section are either closely linked or dependent on the outcome(s) of the previous procedure(s). Moreover, some procedures require physical efforts that could possibly interfere with other procedures planned during the same visit. Therefore, the sequence of the procedures as described for each visit needs to be strictly followed.

### 9.1.1. Pre-Trial Visit

The Pre-Trial Visit aims at informing the subject appropriately on the clinical trial rationale, objectives and constraints and on his/her rights when accepting to participate into the clinical trial (GCP requirements). Subject will also give his/her explicit consent to allow to use his/her data after being informed on the identity of the controller, the goals of the data treatment, the fair processing of his/her data, and on his/her data subjects rights (EU General Data Protection Regulation [GDPR] requirements). It is the Investigator's responsibility to ensure that each potential subject has the ability to provide adequate and informed consent, understands the nature and the scope of the clinical trial, and has signed informed consent prior to any participation in any phase of this clinical trial, including screening evaluations. The Investigator will also verify that an appropriate washout period has been respected for medication that the subject is using prior to trial participation, if applicable.

At Pre-Trial Visit, the following trial procedures will be performed:

1. Inform the subject, orally and in writing, about the purpose, procedures, and general risks of participating in the clinical trial. Answer his/her questions until he/she is satisfied with the information.
2. Obtain his/her informed consent and GDPR explicit consent in writing.
3. Give a copy of subject information and signed informed consent to the subject.

Only after having obtained the subject's informed consent in writing, the next assessments can be performed:

4. Assign subject Screening ID (Section 8.4.5).
5. Check the washout period for ICS*.
6. Schedule the Screening Visit*.
7. Instruct the subject to come at Screening Visit fasted (for at least 8 hours)*.
8. Instruct the subject to come at Screening Visit without having taken any as-needed medications during the 4 hours before the visit. If this administration is necessary for the subject, he/she should inform the trial site and the visit should be rescheduled accordingly.

*Pre-Trial Visit might be performed at the same time as the Screening Visit if the ICS washout period is ≥ 60 days and if the subject has been fasting for at least the last 8 hours. Otherwise, the Screening Visit should be planned as soon as all these conditions are met, so at maximum 60 (+2) days after the Pre-Trial Visit. The subject should then be instructed to come at Screening Visit fasted (for at least 8 hours).

### 9.1.2. Screening Visit

The procedures of this Screening Visit should be performed at least 4 hours after the subject has taken any as-needed reliever medications and the subject should come fasted (for at least 8 hours). If this is not the case, the visit should be rescheduled accordingly.

At Screening Visit, the following trial procedures will be performed:

1. Review and document inclusion and exclusion criteria (Section 8.4).
2. Record demographic information (Section 9.2.4.1).
3. Check the fasting status of the subject.
4. Obtain the medical and surgical history (Section 9.2.4.2).
5. Obtain the respiratory and asthma history (Section 9.2.4.3).
6. Obtain the medication history (prior medication), including respiratory medication and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.1, and Section 8.8.2 for forbidden medications).
7. Perform a physical and clinical examination (Section 9.2.1.3), including COVID-19 symptoms assessment[21].
8. Perform the following assessments needed to collect all missing inclusion and exclusion criteria information (perform these investigations in the mentioned order and do not proceed with the next one if subject's exclusion is decided based on the previous one):
    a. Measure vital signs (Section 9.2.1.4).
    b. Perform 12-lead ECG (Section 9.2.1.5).
    c. Perform spirometry (Section 9.2.1.7) and measure $FEV_1$.
    d. Collect fasting (8-hour) blood samples for hematology, biochemistry and pregnancy test, and urine samples for urinalysis (Section 9.2.1.2.1).
    e. Perform MCh challenge test if not performed in the last year and if $FEV_1 \geq 70\%$ (Section 9.2.2.1).
    f. Perform spirometry (Section 9.2.1.7):
        i. Measure $FEV_1$.
        ii. If $FEV_1 < 70\%$ or presence of asthma-related clinical symptoms (Section 9.2.1.6), administer as-needed reliever medication (SABA) (Section 9.2.1.8).
    g. Perform a PCR nasopharyngeal swab test for SARS-cov-2 detection
9. Dispense subject diary and give the subject the instructions to complete it over the whole clinical trial (Section 9.2.2.2), including the questionnaire about day- and night-time asthma symptoms, the ACQ-5 and ACT questionnaires to complete in the evening before Visit 1.
10. Schedule Visit 1.
11. Instruct the subject to collect his/her first waking urine between 6 and 9 AM in the morning of the Visit 1 and to write down the collection time in the subject diary and dispense the urine collection bottle.
12. Instruct the subject to come to Visit 1 fasted (for at least 8 hours).
13. Instruct the subject to come to Visit 1 without having taken any as-needed medications during the 4 hours before the visit. If this administration is necessary for the subject, he/she should inform the trial site and the visit should be rescheduled accordingly.

### 9.1.3. Visit 1 – Randomization and Baseline

Visit 1 will take place 5 to maximum 7 days after the Screening Visit, when all laboratory results of the subject have been received.
The Investigator will complete the eligibility assessment of the subject on the basis of the laboratory results (Section 8.4).
The procedures of Visit 1 should be performed at least 4 hours after the subject has taken any as-needed reliever medications.

At Visit 1, the following trial procedures will be performed:

[21] A PCR nasopharyngeal swab test must be performed at screening

1. Obtain prior medication use information and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.1, and Section 8.8.2 for forbidden medications).
2. Perform a physical and clinical examination (Section 9.2.1.3), including local tolerability (Section 9.2.1.6) and COVID-19 symptoms assessments[22].
3. Measure vital signs (Section 9.2.1.4).
4. For female subjects, perform a urine pregnancy test (Section 9.2.1.2.1).
5. If subject's eligibility is confirmed, randomize the subject to assign him/her to a treatment group (Section 8.9).
6. Allocate subject's Randomization ID accordingly (Section 8.4.5).
7. Query the subject as to the occurrence of any safety events observed from Screening Visit to Visit 1 and record any safety events observed or spontaneously volunteered by the subject (Section 9.2.1.1).
8. Collect the first waking urine sample for urinary cortisol/creatinine ratio determination (Section 9.2.1.2.3) and urinalysis (Section 9.2.1.2.1).
9. Collect the diary pages containing the baseline symptom scoring information, i.e. questionnaire about day- and night-time asthma symptoms, the ACQ-5 and ACT questionnaires, the timing of urine collection and check their completion (Section 9.2.2.2).
10. Insert the catheter and collect the blood samples for hematology and biochemistry (Section 9.2.1.2.1), single dose 24-hour PK pre-dose time point (Section 9.2.3.1) and baseline blood eosinophil count (Section 9.2.2.5).
11. Perform FeNO (Section 9.2.2.4).
12. Perform spirometry (Section 9.2.1.7) and measure $FEV_1$, FVC and FIVC.
13. Perform MCh challenge test (determine baseline PC20) (Section 9.2.2.1).
14. Perform spirometry (Section 9.2.1.7):
    a. Measure $FEV_1$, FVC and FIVC.
    b. If $FEV_1 < 70\%$ or presence of asthma-related clinical symptoms (Section 9.2.1.6), administer as-needed reliever medication (SABA) (Section 9.2.1.8).
15. Administer the D0 (single dose 24-hour PK) dose of IMP of treatment period 1 (2 ml, corresponding to 0.250 mg budesonide) (Section 8.5.7).
16. Admit the subject at the trial site for overnight stay.
17. Perform the PK sampling procedure (first post-dose time point = minutes after first dose nebulization, in total 15 blood samples after first IMP dose, over 24 hours; Section 9.2.3.1).
18. Record safety events during the whole stay at the clinical site if occurring and/or reported by the subject (Section 9.2.1.1).
19. At the end of the single dose 24-hour PK sampling, administer the second dose of IMP of treatment period 1 (1 ml, corresponding to 0.125 mg budesonide) (Section 8.5.7).
20. Obtain concomitant medication use since the first IMP administration, i.e. during the whole stay at the trial site, and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.2).
21. Dispense IMPs needed for treatment period 1 to the subject (Section 8.12).
22. Schedule or confirm the next trial visit (Visit 2).
23. Instruct the subject to use his/her SABA medication as needed and to record the use in his/her subject diary (Section 8.8.2).
24. Instruct the subject to complete his/her diary and to bring it to the next visit (Section 9.2.2.2).
25. Instruct the subject to collect his/her first waking urine between 6 and 9 AM in the morning of the next visit and to write down the collection time in the subject diary and dispense the urine collection bottle.
26. Instruct the subject to come to Visit 2 fasted (for at least 8 hours).

[22] A PCR nasopharyngeal swab test may be performed during the study at the investigator's discretion

27. Instruct the subject to come to Visit 2 without having taken any as-needed medications during the 4 hours before the visit. If this administration is necessary for the subject, he/she should inform the trial site and the visit should be rescheduled accordingly.

### 9.1.4. Visit 2

Visit 2 should take place on Day 28 (+2) of the first treatment period. Subject will continue to take IMP in the morning each day before Visit 2.
The procedures of Visit 2 should be performed at least 4 hours after the subject has taken any as-needed reliever medications.

At Visit 2, the following trial procedures will be performed:

1. Query the subject as to the occurrence of any safety events and record any safety events observed or spontaneously volunteered by the subject (Section 9.2.1.1).
2. Collect and review completed subject diary pages (Section 9.2.2.2).
3. Obtain information about concomitant medication use and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.2, and Section 8.8.2 for forbidden medications).
4. Collect the first waking urine sample for urinary cortisol/creatinine ratio determination (Section 9.2.1.2.3) and urinalysis (Section 9.2.1.2.1).
5. Perform drug accountability (Section 8.12).
6. Perform a physical and clinical examination (Section 9.2.1.3), including local tolerability (Section 9.2.1.6) and COVID-19 symptoms assessments[23].
7. Measure vital signs (Section 9.2.1.4).
8. Perform 12-lead ECG (Section 9.2.1.5).
9. Perform FeNO (Section 9.2.2.4).
10. Perform spirometry (Section 9.2.1.7) and measure $FEV_1$, FVC and FIVC.
11. Insert the catheter and collect the blood samples for hematology and biochemistry (Section 9.2.1.2.1), abridged PK pre-dose time point (Section 9.2.3.2) and blood eosinophil count (Section 9.2.2.5).
12. Perform MCh challenge test (determine PC20) (Section 9.2.2.1).
13. Perform spirometry (Section 9.2.1.7):
    a. Measure $FEV_1$, FVC and FIVC.
    b. If $FEV_1 < 70\%$ or presence of asthma-related clinical symptoms (Section 9.2.1.6), administer as-needed reliever medication (SABA) (Section 9.2.1.8).
14. Administer the last dose of IMP of treatment period 1 (1 ml, corresponding to 0.125 mg budesonide) (Section 8.5.7).
15. Perform the abridged PK sampling procedure (first post-dose time point = minutes after end of last dose nebulization, in total 6 blood samples after last dose, over 6 hours).
16. Record safety events during the whole stay at the clinical site if occurring and/or reported by the subject (Section 9.2.1.1).
17. Schedule or confirm the next trial visit (Visit 3) after a washout period of 14 (+2) days.
18. Instruct the subject to use his/her SABA medication as needed and to record the use in his/her subject diary (Section 8.8.2).
19. Instruct the subject to come to Visit 3 with his/her subject diary (Section 9.2.2.2).
20. Instruct the subject to collect his/her first waking urine between 6 and 9 AM in the morning of Visit 3 and to write down the collection time in the subject diary and dispense the urine collection bottle.
21. Instruct the subject to come to Visit 3 fasted (for at least 8 hours).

[23] A PCR nasopharyngeal swab test may be performed during the study at the investigator's discretion

22. Instruct the subject to come to Visit 3 without having taken any as-needed medications during the 4 hours before the visit. If this administration is necessary for the subject, he/she should inform the trial site and the visit should be rescheduled accordingly.

### 9.1.5. Visit 3

Visit 3 should take place within maximum 2 days after the planned date of end of the 14-day washout period.
The procedures of Visit 3 should be performed at least 4 hours after the subject has taken any as-needed reliever medications.

At Visit 3, the following trial procedures will be performed:

1. Query the subject as to the occurrence of any safety events and record any safety events observed or spontaneously volunteered by the subject (Section 9.2.1.1).
2. Query the subject as to the use of concomitant medication and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.2, and Section 8.8.2 for forbidden medications) during the washout period
3. Collect and review completed subject diary page about the timing of urine collection (Section 9.2.2.2).
4. Collect the first waking urine sample for urinary cortisol/creatinine ratio determination (Section 9.2.1.2.3) and urinalysis (Section 9.2.1.2.1).
5. Perform a physical and clinical examination (Section 9.2.1.3), including local tolerability (Section 9.2.1.6) and COVID-19 symptoms assessments[24].
6. Measure vital signs (Section 9.2.1.4).
7. Perform spirometry (Section 9.2.1.7) and measure $FEV_1$ and FVC.
8. Insert the catheter and collect the blood samples for hematology and biochemistry (Section 9.2.1.2.1) and single dose 24-hour PK pre-dose time point (Section 9.2.3.1).
9. Administer the D0 (single dose 24-hour PK) dose of IMP of treatment period 2 (2 ml, corresponding to 0.250 mg budesonide) (Section 8.5.7).
10. Admit the subject at the trial site for overnight stay.
11. Perform the PK sampling procedure (first post-dose time point = minutes after first dose nebulization, in total 15 blood samples after first dose, over 24 hours) (Section 9.2.3.1).
12. Record safety events during the whole stay at the clinical site if occurring and/or reported by the subject (Section 9.2.1.1).
13. At the end of the single dose 24-hour PK sampling, administer the second dose of IMP of treatment period 2 (1 ml, corresponding to 0.125 mg budesonide) (Section 8.5.7).
14. Dispense IMPs of treatment period 2 to the subject (Section 8.12).
15. Schedule or confirm the next trial visit (Visit 4).
16. Instruct the subject to use his/her SABA medication as needed and to record the use in his/her subject diary (Section 8.8.2).
17. Instruct the subject to complete his/her diary and bring it to Visit 4 (Section 9.2.2.2).
18. Instruct the subject to collect his/her first waking urine between 6 and 9 AM in the morning of Visit 4 and to write down the collection time in the subject diary and dispense the urine collection bottle.
19. Instruct the subject to come to Visit 4 fasted (for at least 8 hours).
20. Instruct the subject to come to Visit 4 without having taken any as-needed medications during the 4 hours before the visit. If this administration is necessary for the subject, he/she should inform the trial site and the visit should be rescheduled accordingly.

[24] A PCR nasopharyngeal swab test may be performed during the study at the investigator's discretion

### 9.1.6. Visit 4 – End-of-Trial

Visit 4 (End-of-Trial Visit) should take place on Day 28 (+2) of the second treatment period. Subject will continue to take IMP each day before Visit 4.
The procedures of Visit 4 should be performed at least 4 hours after the subject has taken any as-needed reliever medications.

At Visit 4, the following trial procedures will be performed:

1. Query the subject as to the occurrence of any safety events and record any safety events observed or spontaneously volunteered by the subject (Section 9.2.1.1).
2. Collect and review completed subject diary pages and ensure the full diary has been returned by the subject (Section 9.2.2.2).
3. Obtain information about concomitant medication use and especially the use of SABA (as-needed reliever medication) and any other asthma medications (Section 9.2.4.4.2, and Section 8.8.2 for forbidden medications).
4. Collect the first waking urine sample for urinary cortisol/creatinine ratio determination (Section 9.2.1.2.3) and urinalysis (Section 9.2.1.2.1).
5. Perform drug accountability (Section 8.12).
6. Perform a physical and clinical examination (Section 9.2.1.3), including local tolerability (Section 9.2.1.6) and COVID-19 symptoms assessments[25].
7. Measure vital signs (Section 9.2.1.4).
8. Perform 12-lead ECG (Section 9.2.1.5).
9. Perform FeNO (Section 9.2.2.4).
10. Perform spirometry (Section 9.2.1.7) and measure $FEV_1$, FVC and FIVC.
11. Insert the catheter and collect the blood samples for hematology and biochemistry (Section 9.2.1.2.1), abridged PK pre-dose time point (Section 9.2.3.2) and blood eosinophil count (Section 9.2.2.5).
12. Perform MCh challenge test (determine PC20) (Section 9.2.2.1).
13. Perform spirometry (Section 9.2.1.7):
    a. Measure $FEV_1$, FVC and FIVC.
    b. If $FEV_1 < 70\%$ or presence of clinical symptoms related to asthma (Section 9.2.1.6), administer as-needed reliever medication (SABA) (Section 9.2.1.8).
14. Administer the last dose of IMP of treatment period 2 (1 ml, corresponding to 0.125 mg budesonide) (Section 8.5.7).
15. Perform the abridged PK sampling procedure (first post-dose time point = minutes after end of last dose nebulization, in total 6 blood samples after last dose, over 6 hours).
16. Record safety events during the whole stay at the clinical site if occurring and/or reported by the subject (Section 9.2.1.1).
17. Advise the subject on the most suitable treatment for follow-up, i.e. subsequent therapy counseling (Section 9.2.4.5).

After the final examination, the clinical trial is considered completed for the subject. No further trial-related procedures will be performed, unless safety events require follow-up (Section 9.2.1.1).

### 9.1.7. Early Termination Visit

In case of early termination or withdrawal of subject (Section 10.5.1), the procedures listed in Visit 4 will be performed, if considered safe for the subject by the Investigator and if possible. An end-of-trial form will be completed by the trial staff.
Trial site staff will attempt to follow the progress of every subject admitted to the clinical trial until trial completion. If a subject fails to return for a scheduled visit, a reasonable effort will be made by the trial

[25] A PCR nasopharyngeal swab test may be performed during the study at the investigator's discretion

site staff to contact the subject and ascertain the reason(s) for not returning. As a minimum, the site staff will contact the subject twice by phone and, if no response, will send a letter by registered mail.

If a subject does not complete the clinical trial for any reason (including at Investigator's discretion), the reason and circumstances for the subject's early termination must be fully documented. If possible, the trial procedures specified for the Visit 4 should be performed.

The Sponsor or designee must be informed in a timely manner if any subject withdraws from the trial, regardless of the cause.

#### 9.1.8. Unscheduled Visit

If deemed necessary for the patient's safety and well-being, the Investigator can initiate an unscheduled visit. All such visits will be documented in the eCRF with any additional required documentation based on the nature of the unscheduled visit.

#### 9.1.9. Follow-up Contact

In order to collect any post-trial SAEs, the Investigator will contact each subject 30 (+2) days after his/her last visit, regardless of whether the subject has completed the clinical trial or has been withdrawn before the end of the trial.
In case a post-trial SAE is identified, it should be entered into the eCRF within 24 hours after becoming aware of the information. In case the eCRF is not available, the safety information should be entered in the SAE Report Form and submitted to Clinfidence pharmacovigilance department within 24 hours after becoming aware of the information (Section 9.2.1.1.2).

## 9.2. Assessments and their Appropriateness

### 9.2.1. Safety Assessments

#### 9.2.1.1. *Assessments for Safety Events*

All of the following safety information (safety events) will be collected:

- AEs, SAEs, and post-trial SAEs
- Pregnancies, IMP overdose, drug interaction, medication error.

All safety events observed during the trial from the time the subject signs the informed consent until the last contact will be collected.

##### *9.2.1.1.1. Adverse Events and Adverse Reactions*

Definitions

Adverse event (AE): An AE is any untoward medical occurrence in a trial subject receiving an IMP and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an IMP, whether related to the IMP or not.

Adverse reaction (AR): ARs are all untoward and unintended responses to an investigational product related to any dose administered.

Other significant AEs: Any marked laboratory abnormalities or any AEs that lead to an intervention, including withdrawal of IMP treatment, dose reduction, or significant additional concomitant therapy.

Withdrawal due to AE/AR: AE/AR leading to discontinuation of treatment with IMP. Any such events will be followed up by the Investigator until the event is resolved or until the medical condition of the subject is stable. All follow-up information collected will be made available to the Sponsor.

Collection of AEs

The condition of the subject will be monitored throughout the clinical trial. At each visit, whether scheduled or unscheduled, AEs will be elicited using a standard nonleading question such as "How have you been since the last visit/during the previous trial period?" In addition, the Investigator will check the subject diaries for any documented event.

Any AE or AR, which occurs during the clinical trial, at trial site or reported to the home-visiting study nurses, will be noted in detail in the eCRF. If the subject reports several signs or symptoms representing a single syndrome or diagnosis, the diagnosis should be recorded in the eCRF. The Investigator will grade the severity of all AEs or ARs (mild, moderate, or severe), the seriousness (nonserious or serious), and the causality (probable, possible, unlikely, not related or unclassified).

In the event of clinically significant abnormal laboratory findings, the test results will be confirmed, and the subject followed up until the laboratory values have returned to normal and/or an adequate explanation for the abnormality has become available.

Diseases, signs and symptoms, and/or laboratory abnormalities already present before the first administration of IMP will not be considered AEs unless an exacerbation in intensity or frequency (worsening) occurs.

The Investigator will provide detailed information about any abnormalities and about the nature of and reasons for any action taken as well as any other observations or comments that may be useful for the interpretation and understanding of an AE or AR.

Asthma exacerbations

Asthma exacerbations will be considered as adverse if they reflect, to the opinion of the Investigator, a worsening of the subject's disease. To allow this evaluation, all asthma exacerbations will be reported as AEs, as well as the treatment (concomitant medication) administered and a report, issued by the data management department or the pharmacovigilance department, will be sent to the Investigator on a weekly basis, according to the instructions described in the safety management plan.

Severity of AEs

The intensity/severity of AEs will be graded as follows:

- Mild: an AE, usually transient, which causes discomfort but does not interfere with the subject’s routine activities,
- Moderate: an AE which is sufficiently discomforting to interfere with the subject’s routine activities,
- Severe: an AE which is incapacitating and prevents the pursuit of the subject's routine activities.

The grading of an AE is up to the medical judgement of the Investigator and will be decided on a case-by-case basis.

Causality of AEs

The relationship of AEs to the administered IMP will be assessed by the Investigator:

- Probable: reports including good reasons and sufficient documentation to assume a causal relationship, in the sense of plausible, conceivable, likely, but not necessarily highly probable. A reaction that follows a reasonable temporal sequence from administration of the IMP; or that follows a known or expected response pattern to the suspected medicine; or that is confirmed by stopping or reducing the dosage of the medicine and that could not reasonably be explained by known characteristics of the subject's clinical state,
- Possible: reports containing sufficient information to accept the possibility of a causal relationship, in the sense of not impossible and not unlikely, although the connection is uncertain or doubtful, for example because of missing data or insufficient evidence. A reaction that follows a reasonable temporal sequence from administration of the IMP; that follows a known or expected response pattern to the suspected medicine; but that could readily have been produced by a number of other factors,
- Unlikely: reports not following a reasonable temporal sequence from IMP administration. An event which may have been produced by the subject's clinical state or by environmental factors or other therapies administered,
- Not related (unrelated): events for which sufficient information exists to conclude that the etiology is unrelated to the IMP,
- Unclassified: reports which for one reason or another are not yet assessable, e.g., because of outstanding information (can only be a temporary assessment).

Product-related causality (ARs) will be defined as events that are probably related or possibly related to the IMP. Events will be categorized as unrelated to IMP if they are reported as unlikely, unclassified or not related.

Outcome of AEs

The outcome of all reported AEs has to be documented as follows:

1. Recovered, resolved
2. Recovering, resolving
3. Not recovered, not resolved
4. Recovered, resolved with sequelae
5. Fatal
6. Unknown

A subject's death *per se* is not an event, but an outcome. The event which resulted in the subject's death must be fully documented and reported, even in case the death occurs within 30 days after IMP treatment end and regardless of whether or not it is considered treatment related.

Action(s) taken with subject

AEs requiring action or therapy will be treated with recognized standards of medical care to protect the health and well-being of the subject. Appropriate resuscitation equipment and medicines will be available to ensure the best possible treatment in case of an emergency.
The actions taken with each subject by the Investigator will be documented.
The Investigator will follow up on each AE until it has resolved or until the medical condition of the subject has stabilized. Any follow-up information will be reported to the Sponsor.
The Investigator can withdraw the subject from the clinical trial for safety reasons (Section 10.5.1).

Action(s) taken with IMP under investigation

As described in Section 10.1.2, Sponsor is responsible for the ongoing safety evaluation of the IMP under investigation and will expedite the notification to the Principal Investigator and regulatory authorities of findings that are both serious and unexpected (Section 9.2.1.1.2) and/or that could adversely affect the safety of subjects, the conduct of the clinical trial or alter the Independent Ethics Committee (IEC)/Institutional Review Board (IRB)'s approval to continue the clinical trial.
Significant worsening of the benefit/risk ratio of AQ001S 0.125 mg/ml will lead to reevaluate the appropriateness to continue, hold or stop the clinical trial (Section 10.6.1.1).

Documentation of AEs

All AEs that occur during the trial are to be recorded in the eCRF. The Investigator should complete all the details requested, including dates of onset, time of onset, stop date (when applicable), stop time (when applicable), severity, action taken, outcome, and relationship to IMP and to trial procedures.

#### *9.2.1.1.2. Serious Adverse Events*

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

- Results in death,
- Is life-threatening (see below),
- Requires hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity,
- Is a congenital anomaly/birth defect.

Any other important medical event that may not result in death, be life-threatening or require hospitalization may be considered an SAE when, based upon appropriate medical judgement by the Principal Investigator, the event may jeopardize the participant or may require an intervention to prevent one of the other serious outcomes listed above.

NOTE: The term 'life-threatening' refers to an event in which the subject was, in the view of the reporting Investigator, at immediate risk of death at the time of the event; it does not refer to an event which may hypothetically have caused death had it been more severe.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

Unexpected ARs are SUSARs if the following three conditions are met:

1. The event must be serious,
2. There must be a certain degree of probability that the event is a harmful and an undesirable reaction to the IMP, regardless of the administered dose,
3. The AR must be unexpected, that is to say, the nature and severity of the AR are not in agreement with the product information as recorded in the IB (Section 8) or other reference safety information of the IMP.

Should a SUSAR occur, it will be reported to the IEC/IRB and to the applicable authorities in accordance with Directive 2001/20/EC.

SAE Reporting Timelines

All SAEs, whether or not they are suspected to be related to the IMPs, should be entered into the eCRF within 24 hours of becoming aware of the information, which in turn will trigger a notification to department which will follow up and contact the site.

In case the eCRF is not available, the safety information should be entered on the SAE Report Form and submitted to department within 24 hours of becoming aware of the information using the following email address:

| **Email**: |
|---|

In case the site has no access to internet, the SAE can be reported by telephone using the following telephone number:

| **Telephone**: |
|---|

Surgeries that are elective or were planned before clinical trial entry are not considered (S)AEs and not required to be reported.

Post-trial SAE reports

Any SAE which occurs up to 30 (+2) days after the last IMP administration should be reported by the Investigator to the Sponsor within 24 hours of becoming aware of the information. Proactive monitoring for post-trial SAEs will be done by the Investigator by phone (Section 9.1.9).

#### *9.2.1.1.3. Other Relevant Safety Information*

Pregnancies

Every effort will be made to avoid a pregnancy during trial enrollment and trial conduct (serum pregnancy test performed at Screening Visit, urine pregnancy test performed at Visit 1 and inclusion of female subjects of childbearing potential only if they use a highly effective contraception method for 28 days before the trial, during the trial and for 28 days after the end of the trial). In case of pregnancy occurring during the clinical trial, the subject will be discontinued of IMP treatment and withdrawn from the clinical trial.
Pregnancies occurring during the clinical trial and exposing the fetus to the IMP need to be recorded and reported.
In case of such an event, the Investigator should complete the pregnancy notification form and send it to the clinical project manager or designee according to the instructions described in the safety management plan.
Upon subject's consent, the Investigator will collect all follow-up information on the outcome of both mother and fetus up to childbirth and send it to a Sponsor representative.

The following safety relevant information should be reported as an AE or, if the reaction fulfils one of the criteria for seriousness, as an SAE.

Drug overdose

An overdose is a deliberate or inadvertent administration of a treatment at a dose higher than specified in the protocol and higher than the known therapeutic dose that is of clinical relevance. The overdose *per se* should not be reported as an AE. If adverse, the reaction (signs, symptoms, clinical abnormalities) of a suspected overdose of either the IMP or a concomitant medication must be clearly identified and

reported as an AE. In case of suspected overdose, the patient should be treated according to standard medical practice based on the Investigator's judgment.

Drug interaction

A drug interaction is a situation in which a substance or medicinal product affects the activity of an IMP, i.e., increases or decreases its effects, or produces an effect that none of the products would exhibit on its own. The reaction of a suspected drug interaction must be clearly identified and reported as an AE.

Medication error

A medication error involves the inadvertent administration or unintended use of a medicinal product which may be caused by the naming, presentation of pharmaceutical form/packaging or IFU/labelling. The reaction must be clearly identified as a medication error.

#### 9.2.1.2. *Clinical Laboratory Tests*

##### *9.2.1.2.1. Hematology, Biochemistry, Urinalysis and Pregnancy Tests*

Clinical laboratory tests include blood analysis (hematology and biochemistry) and urinalysis.
Subject should be fasting for at least the last 8 hours before blood collection, particularly for the Screening Visit to assess eligibility of the subject (Section 9.1.2).
Clinical laboratory tests will be performed at the time points/visits indicated in the flowchart in Section 3. On dosing days at trial site, the assessments will be done according to the sequence described in the visit procedures (Section 9.1).

A local laboratory will perform the classical clinical laboratory tests on blood samples, including serum pregnancy test for female subjects of childbearing potential allowing to exclude pregnant subjects from entering the clinical trial.

For urinalysis, test strip analyses will be performed on the first waking urine samples at the trial site at the time of sample collection. If a test result is suspicious/unsure, a urine sample will be sent to the local laboratory for in-depth urinalysis with the optional tests listed in Appendix 3.
At Visit 1, a confirmatory urine pregnancy test will be performed at the trial site for female subjects of childbearing potential allowing to exclude pregnant subjects from receiving the IMPs.

The appended table (Appendix 3) lists all laboratory tests to be performed, normal values and reasons to test.
Blood and urine samples will be prepared, and shipments made to the laboratory, according to their recommendations, and sample storage conditions will be documented. Only sample collection date and time must be entered in the eCRF.

The methods of laboratory test determination will be provided in the clinical study report. These methods are validated methods used in clinical routine by the laboratory.

All out-of-range laboratory values that are considered by the Investigator to be clinically significant will be recorded as AEs (Section 9.2.1.1.1).

The laboratory that will perform the sample analyses is specified in Section 1.

##### *9.2.1.2.2. Amounts and Combination of Blood and Urine Collections*

The estimated amount of blood to be collected per visit, including all blood/plasma assessments is, for each subject:

- A total of 24.5 ml of blood at Screening Visit (for eligibility criteria),
- A total of 72.5 ml of blood at Visit 1 (24.5 ml for hematology and biochemistry, including blood eosinophil count, and 45 ml for full single 24-hour PK),
- A total of 45.5 ml of blood at Visit 2 (24.5 ml for hematology and biochemistry, including blood eosinophil count, and 21 ml for abridged PK),
- A total of 72.5 ml of blood at Visit 3 (24.5 ml for hematology and biochemistry, and 45 ml for full single 24-hour PK),
- A total of 45.5 ml of blood at Visit 4 (24.5 ml for hematology and biochemistry, including blood eosinophil count, and 21 ml for abridged PK).

The estimated total maximum blood volume needed per subject during the trial (when completing the trial until the last visit) is 260.5 ml, over 77 to 85 days, from Screening Visit until Visit 4 (End-of-Trial).

At Screening Visit, blood and urine samples will be respectively collected with classical venipuncture and urine collection materials.
At Visits 1, 2, 3 and 4, the blood and urine collections for hematology, biochemistry and urinalysis will be combined with those necessary for the blood PK sampling (Section 9.2.3) and eosinophil count (Section 9.2.2.5), i.e. through a catheter, and for the urinary cortisol/creatinine ratio, i.e. in the first waking urine (Section 9.2.1.2.3).

#### *9.2.1.2.3. Assessment of Hypothalamic Pituitary Adrenocortical Axis Function*

Hypothalamic pituitary adrenocortical (HPA) axis function will be assessed through specific urinalysis at the time points/visits indicated in the flowchart in Section 3. On dosing days at trial site, the assessments will be done according to the sequence described in the visit procedures (Section 9.1).
For that purpose, the urinary cortisol/creatinine ratio will be determined in first waking urine collected by the subject between 6 and 9 AM.
The local laboratory that will determine the cortisol/creatinine ratio analysis in the urinary samples is specified in Section 1.
Population reference values for the urinary cortisol/creatinine ratio in first waking urine for urine collection between 6 and 9 AM have been established by the laboratory prior to the clinical trial (Appendix 3). The methods of cortisol/creatinine ratio determination will be provided in the clinical study report. Urine samples will be prepared, and shipments made to the laboratory in charge of the cortisol/creatinine ratio determination according to their recommendations and the storage conditions will be documented. The timing of first waking urine collection will be recorded by the subjects in their diary (Section 9.2.2.2).

All out-of-range laboratory values that are considered by the Investigator to be clinically significant will be recorded as AEs (Section 9.2.1.1).

### 9.2.1.3. *Physical and Clinical Examination*

The physical and clinical examination include height (only measured at Screening Visit), weight, body mass index (calculated), oropharyngeal examination and covid-19 symptoms assessments by the Investigator (physician), and will be recorded at the time points/visits indicated in the flowchart in Section 3. On dosing days at trial site, the examinations will be done according to the sequence described in the visit procedures (Section 9.1).
The oropharyngeal examination will include the investigation of specific ICS-related signs, such as vocal cord myopathy and fungal infection.

All abnormal findings in physical and clinical examination that are considered by the Investigator to be clinically significant will be recorded as AEs (Section 9.2.1.1).

#### 9.2.1.4. *Vital Signs*

Vital signs will be measured at the time points/visits indicated in the flowchart in Section 3. On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).
Vital signs will include records of blood pressure (seated), body temperature, heart rate, and blood oxygen saturation. Vital sign measurements will be taken while the subject is seated after at least 5 minutes at rest.
Systolic and diastolic blood pressure measurements will be made using a sphygmomanometer with a cuff size appropriate for each individual subject.
Heart rate and blood oxygen saturation will be measured with a pulse oximeter.
Temperature will be measured orally.

All abnormal findings in vital signs that are considered by the Investigator to be clinically significant will be recorded as AEs (Section 9.2.1.1).

#### 9.2.1.5. *Twelve-Lead Electrocardiogram*

Single 12-lead paper ECG will be obtained at the time points/visits indicated in the flowchart in Section 3. On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).
The single 12-lead ECG will be recorded while subject is in resting position (supine) for at least 5 minutes. For each ECG, the following parameters will be calculated and recorded into the eCRF:

- Heart rate,
- QT interval duration,
- corrected QT interval (QTc), estimating the QT interval at a standard heart rate of 60 beat per minute.

Twelve-lead ECGs will be interpreted by the Investigator at the site as soon as possible after the time of ECG collection, and ideally while the subject is still present at trial site, for immediate subject management.
All abnormal findings in ECGs that are considered by the Investigator to be clinically significant will be recorded as AEs (Section 9.2.1.1).

#### 9.2.1.6. *Local Tolerability*

Local tolerability will be assessed at the time points/visits indicated in the flowchart in Section 3 through physical and clinical examination (Section 9.2.1.3). On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).

In particular, the following specific parameters will be reported:

- Increased bronchial irritability,
- Paradoxical bronchospasm,
- Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection).

The presence of asthma-related clinical symptoms (e.g. dyspnea, cough, wheezing, bronchospasm, etc.) after MCh challenges, along with the $FEV_1$ spirometry measurements at Visits 1, 2 and 4, will determine the need to administer as-needed reliever medication (SABA) to the subject (if $FEV_1 < 70\%$; Section 9.2.1.8).

#### 9.2.1.7. *Spirometry*

Spirometry, a common pulmonary function test, will be performed according to the American Thoracic Society/European Respiratory Society standardization of spirometry [51]. The following parameters will be measured at the time points/visits indicated in the flowchart in Section 3:

- Forced expiratory volume at 1 second ($FEV_1$) to check eligibility, as safety parameter and as efficacy parameter,
- Forced vital capacity (FVC) as safety parameter,
- Forced inspiratory vital capacity (FIVC) as efficacy parameter.

$FEV_1$ and FIVC are indeed meant to be improved by an asthma treatment.

Therefore, spirometry will be performed at the indicated visits in order to:

- Measure $FEV_1$ to check inclusion criteria: Screening Visit,
- Measure $FEV_1$, FVC and FIVC before and after MCh challenge test for safety and efficacy assessments (baseline values): Visit 1,
- Measure $FEV_1$, FVC and FIVC before and after MCh challenge test for safety and efficacy assessments (end-of-treatment period values): Visits 2 and 4,
- Measure $FEV_1$ and FVC for safety assessments: Visit 3.

On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).

The $FEV_1$ measurements before and after MCh challenge tests allow to determine the PC20 as described in Section 9.2.2.1 and according to the guidelines of the American Thoracic Society [41].

The $FEV_1$ measurements after MCh challenge test will determine, along with the presence of asthma-related clinical symptoms (Section 9.2.1.6), the need to administer as-needed reliever medication (SABA) to the subject (if $FEV_1 < 70\%$; Section 9.2.1.8).

#### 9.2.1.8. *Administration of As-Needed Reliever Medication*

The administration of as-needed reliever medication (SABA) at any time of the clinical trial will be recorded, including by the subject in his/her diary on a daily basis.

At Visits 1, 2 and 4, the $FEV_1$ measurements after MCh challenge tests (Section 9.2.1.7) and the recording of asthma-related clinical symptoms (Section 9.2.1.6) will determine the need to administer as-needed reliever medication (SABA) to the subject (if $FEV_1 < 70\%$ or presence of asthma clinical symptoms).

### 9.2.2. Efficacy and Pharmacodynamic Assessments

#### 9.2.2.1. *Methacholine Challenge Test and PC20*

In the present clinical trial, MCh challenge tests will be performed at the time points/visits indicated in the flowchart in Section 3, using a marketed MCh powder (Section 8.6) as a provocative agent (i.e. the auxiliary medicinal product [AxMP] Provocholine®). On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).

The following protocol, procedures and sequence described in the American Thoracic Society guidelines [41] will be followed to perform this MCh challenge test.

Protocol:
A continuous 2-minute tidal breathing dosing protocol will be used in the present clinical trial.

Preparation of the MCh concentrations:
The following 4 concentrations of MCh in sterile vials using a sterile solution of NaCl 0,9% as diluent must be prepared, placed in a holder, and stored in a refrigerator (2 to 8°C). These solutions can be stored for not more than 2 weeks in a refrigerator.

Diluent: sterile NaCl 0.9% solution for injection
MCh concentrations: 0.25, 1, 4 and 16 mg/ml (see Table 2)

**Table 2 - Provocholine® powder dilutions (one 100-mg vial of methacholine chloride) with 0.9% NaCl injection in sterile vials**

| Label strength | Take | Add NaCl 0.9% (diluent) | Total volume | Dilution factor | MCh dilution X | MCh dilution concentration |
|---|---|---|---|---|---|---|
| 100 mg | 100 mg | 6.25 mL | 6.25 mL | N/A | Dilution A | 16 mg/mL |
| | 3 mL of Dilution A | 9 mL | 12 mL | 1:4 | Dilution B | 4 mg/mL |
| | 3 mL of Dilution B | 9 mL | 12 mL | 1:4 | Dilution C | 1 mg/mL |
| | 3 mL of Dilution C | 9 mL | 12 mL | 1:4 | Dilution D | 0.25 mg/mL |

Rationale for the concentration's selection:
The above-mentioned MCh concentrations were selected for the present clinical trial because of concerns about the safety of the 10-fold changes in dilution strengths described in the protocol of Provocholine® IFU. Quadrupling concentration increments are recommended by the guidelines of the American Thoracic Society [41].

Preparation of the subject for MCh challenge test:
- The test must be explained to the subject. The subjects should be told they may experience some minor symptoms, such as cough or chest tightness, but that most subjects have no symptoms. They should be warned that occasional severe symptoms may occur.
- The subject should be asked if they would like to urinate before the test (stress incontinence could be precipitated, especially in older women).
- The subjects should be evaluated for contraindications and medication use should be reviewed.

Dosage and administration:
- The MCh dilution vials should be removed from the refrigerator 30 min before testing, so that the mixture warms to room temperature before use.
- Before MCh challenge test is begun, baseline pulmonary function tests must be performed inserting 3 mL of diluent (i.e., sterile solution of NaCl 0.9% for injection) into the nebulizer, using a sterile syringe. A subject to be challenged must have an $FEV_1$ of at least 70% of the predicted value.
- The MCh challenge test is performed by giving the subject ascending serial concentrations of Provocholine® (i.e., starting from dilution D [0.25 mg/mL], dilution C [1 mg/mL], dilution B [4 mg/mL] and ending by dilution A [16 mg/mL] subsequently).
- During the MCh challenge test, the subject should be instructed to relax and breathe quietly (tidal breathing) for 2 min. The timer must be set for 2 min. A noseclip may be applied.
- 3 ml of the appropriate dilution must be inserted into the nebulizer, using a sterile syringe.
- $FEV_1$ must be measured about 30 and 90 s after the nebulization of each serial concentration is completed. An acceptable-quality $FEV_1$ must be obtained at each time point. To keep the

cumulative effect of MCh relatively constant, the time interval between the commencement of two subsequent concentrations should be kept to 5 min.
- If the $FEV_1$ falls less than 20%, empty the nebulizer (any remaining solution should be discarded) and repeat the nebulization with the inhalation of 3 ml of the next highest MCh concentration.
- If the $FEV_1$ falls more than 20% from baseline (or the highest MCh concentration [i.e., dilution A] has been given), no further MCh must be given. The signs and symptoms experienced by the subject must be noted. Inhaled as-needed reliever medication (SABA) must be administered. Spirometry must be repeated after 10 minutes.
- The PC20 $FEV_1$ must be calculated by linear interpolation based on the antepenultimate Provocholine® dilution and on the last Provocholine® dilution that induced a $FEV_1$ that fell more than 20% from baseline.
- The PC20 must be recorded in the eCRF.

To ensure the quality of the tests, subjects must not ingest caffeine-containing products and must be under fasting conditions at the visits during which these tests are performed.

At Visit 1 (baseline), Visit 2 and Visit 4, a MCh challenge test will be performed, i.e. up to the administration of a concentration of MCh provoking an $FEV_1$ fall of 20% (PC20).

#### 9.2.2.2. *Subject Diaries and Questionnaires*

Subject diaries will be given to the participating subjects (in their native language) at the time points/visits indicated in the flowchart in Section 3. Subjects will be instructed on how to complete their diary by the Investigator or designee.

The following information will be recorded by the subject in his/her diary:
- Symptom questionnaires and scores will be answered by the trial subjects:
    a. Daily questionnaire: day- and night-time asthma symptom scoring,
    b. Weekly Asthma Control Questionnaire™ (ACQ-5) questionnaire,
    c. Monthly Asthma Control Test™ (ACT) questionnaire.
- Use of as-needed reliever medication (daily),
- Use of concomitant medications (weekly),
- Time of first waking urine collection in the morning of Visits 1 to 4.

The daily questionnaire is based on questions published in the literature [52].

The ACQ-5 is a 5-item, subject-based simple questionnaire to measure the adequacy of asthma control and change in asthma control which occurs either spontaneously or as a result of treatment. It is a clinically validated assessment of asthma control [53].

The ACT is a brief, 5-item subject-based assessment designed to measure dimensions of asthma control outlined in the current asthma management guidelines as defined by the National Heart, Lung, and Blood Institute: asthma symptoms, use of as-needed reliever medications, and the impact of asthma on everyday functioning. It is suitable for use with or without lung-function testing [54].

At the time points/visits indicated in the flowchart in Section 3, the completed subject diary pages will be collected and reviewed by the Investigator.

#### 9.2.2.3. *Spirometry*

See Section 9.2.1.7.

#### 9.2.2.4. *Fractional Concentration of Exhaled Nitric Oxide*

The fractional concentration of exhaled nitric oxide (FeNO) is an airway inflammation PD biomarker. It will be measured before MCh challenge tests at the time points/visits indicated in the flowchart in Section 3 using the FeNO+ system of Medisoft®. For a proper FeNO measurement, subjects must not eat nitric oxide-rich food 3 hours before the test, but they will be fasting for at least 8 hours before the visits including this assessment which should prevent any interference with the test. On dosing days at trial site, the measurements will be done according to the sequence described in the visit procedures (Section 9.1).

#### 9.2.2.5. *Blood Eosinophil Count*

The blood eosinophil count is an airway inflammation PD biomarker. It will be calculated at the time points/visits indicated in the flowchart in Section 3 from the hematology results (Section 9.2.1.2.1). Only the results of Visits 1, 2 and 4 will be considered in the efficacy analysis (results of Visit 3 will only be considered as safety results).
The amount of blood to be collected at each visit, including all blood/plasma assessments is described in Section 9.2.1.2.2.

### 9.2.3. Pharmacokinetic Assessments

Blood samples will be collected for measuring budesonide plasma concentrations at the time points/visits indicated in the flowchart in Section 3, i.e.:

- Visits 1 and 3 for full single 24-hour PK,
- Visits 2 and 4 for abridged PK.

#### 9.2.3.1. *Single Dose 24-Hour PK Sampling*

The single dose 24-hour PK corresponds to the first dose administration, i.e. 0.250 mg of AQ001S or the comparator, on D0 of each treatment period (at the time points/visits indicated in the flowchart in Section 3). On dosing days at trial site, the blood collections will be done according to the sequence described in the visit procedures (Section 9.1).

Subjects will come to the trial site at Visits 1 and 3 and will stay overnight in order to perform 24-hour PK sampling, in addition to the other procedures detailed in the Visit 1 and Visit 3 sections (Sections 9.1.3 and 9.1.5). For PK sampling, a catheter will be inserted allowing the collection of:

- 1 pre-dose blood sample before IMP administration,
- 15 post-dose blood samples at , , , 20, 30, 45, 60, 90, 120, 180, 240, 360 minutes and 10, 18, 24 hours after IMP administration.

A total of 16 blood samples will be taken, corresponding to 48 ml of blood. The amount of blood to be collected at each visit, including all blood/plasma assessments is described in Section 9.2.1.2.2.

After the pre-dose blood sampling, the subjects will receive their assigned IMP, i.e. either one single dose of 2 ml of AQ001S 0.125 mg/ml (total dose of 0.250 mg budesonide) or one single dose of 2 ml of Budesonide 0.125 mg/ml (total dose of 0.250 mg budesonide), by nebulization (Section 8.5.7).

The time of IMP administration and blood sampling will be recorded.

#### 9.2.3.2. *Abridged PK Sampling*

The abridged PK corresponds to the last dose administration, i.e. 0.125 mg of AQ001S or the comparator, on the last day (D28 [+2] days) of each treatment period (at time points/visits indicated in the flowchart in Section 3). On dosing days at trial site, the blood collections will be done according to the sequence described in the visit procedures (Section 9.1).

Subjects will come to the trial site at Visits 2 and 4 and will stay for approximately 12 hours in order to perform the abridged PK sampling, in addition to the other procedures detailed in the sections dedicated to Visit 2 and Visit 4 (Sections 9.1.4 and 9.1.6, respectively). For PK sampling, a catheter will be inserted allowing the collection of:

- 1 pre-dose blood sample before IMP administration,
- 6 post-dose blood samples at , , , 120, 240 and 360 minutes after IMP administration.

A total of 7 blood samples will be taken, corresponding to 21 ml of blood. The amount of blood to be collected at each visit, including all blood/plasma assessments is described in Section 9.2.1.2.2.

After the pre-dose blood sampling, the subjects will receive their assigned IMP, i.e. either AQ001S or the comparator, corresponding for both formulations to a total dose of 0.125 mg budesonide, by nebulization (Section 8.5.7).

The time of IMP administration and blood sampling will be recorded.

#### 9.2.3.3. *Processing and Bioanalysis of the PK Samples*

Blood samples will be centrifuged in order to recover the plasma, and plasma samples will be stored until analysis. Budesonide plasma concentrations will be determined in these samples by a bioanalysis laboratory (identified in Section 1). Sample processing, storage and shipments to the bioanalysis laboratory will be done according to their recommendations and storage conditions will be documented.

The methods of determination of budesonide in human plasma will be provided in the clinical study report. This specific method will be validated by the dedicated laboratory prior to bioanalysis with a target lower limit of quantification of 10 pg/ml.

The budesonide concentration will be measured in all collected plasma samples for each subject.

#### 9.2.3.4. *Pharmacokinetic Parameters*

The following PK parameters will be calculated based on the budesonide plasma concentrations obtained from bioanalysis, for each treatment period:

- Start of treatment period:
  - single dose 24-hour PK parameters at the time of the first scheduled IMP administration (16 time points): $C_{max}$, $t_{max}$, $C_{last}$, $t_{last}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$, CL/F, V/F, $\lambda_z$ and $t_{1/2z}$,
- End of treatment period:
  - abridged PK at the time of the last scheduled IMP administration (7 time points): $C_{through}$, $C_{max}$, $t_{max}$, $C_{last}$, $t_{last}$, and $AUC_{0\text{-}last}$, $AUC_{0\text{-}6h}$.

<u>PK parameter definitions:</u>

- $C_{max}$: maximum observed plasma budesonide concentration after dosing or during a dosing interval (ng/ml),
- $t_{max}$: time to reach the maximum observed plasma budesonide concentration (h),
- $C_{last}$: last observed plasma budesonide concentration after dosing (ng/ml),
- $t_{last}$: time to reach the last observed plasma budesonide concentration after dosing (h),

- $\lambda_z$: apparent first-order rate constant associated with the terminal slope of the semi-logarithmic budesonide concentration-time curve,
- $t_{1/2z}$: terminal elimination half-life associated with the terminal slope ($\lambda_z$) of the semi-logarithmic budesonide concentration-time curve,
- $AUC_{0\text{-}6h}$: area under the plasma concentration-time curve from time 0 to 6-hours post administration (ng.h/ml),
- $AUC_{0\text{-}last}$: area under the plasma concentration-time curve from time 0 to the last quantifiable concentration (ng.h/ml),
- $AUC_{0\text{-}inf}$: area under the plasma concentration-time curve from time 0 to infinity (ng.h/ml),
- CL/F: apparent clearance following budesonide inhalation (l/h),
- V/F: apparent volume of distribution following budesonide inhalation (l),
- $C_{trough}$: plasma budesonide concentration at the end of a dosing interval, immediately before next IMP administration (ng/ml).

## 9.2.4. Other Assessments

### 9.2.4.1. *Demographics*

The demographic and baseline characteristics include sex and age and will be recorded at Screening Visit.

### 9.2.4.2. *Medical and Surgical History*

Medical and surgical history of the subject will be obtained by interviewing the subject. Clinically significant findings as well as pre-existing conditions present prior to screening will be recorded at Screening Visit (see also inclusion/exclusion criteria in Section 8.4).
This includes a review of:

- All body systems,
- Previous surgical intervention(s), including indication, date and outcome.

### 9.2.4.3. *Respiratory and Asthma History*

Respiratory and asthma history, obtained by interviewing the subject, will be recorded at the time points/visits indicated in the flowchart in Section 3 (see also inclusion/exclusion criteria in Section 8.4).
This includes:

- Date of asthma diagnosis, including date of appearance of first symptoms,
- Asthma exacerbations in the last year,
- Other respiratory history: COPD, bronchiectasis, pulmonary malformations, tuberculosis, cystic fibrosis.

### 9.2.4.4. *Prior/Concomitant Medications*

Information about prior and concomitant medications will be obtained by interviewing the subject and recorded weekly by the subject in the subject diary (Section 9.2.2.2).

Medications with a start date occurring before the first IMP dose date will be identified as prior medications.
The following medications will be identified as concomitant medications :

- Medications with a start date occurring on or after the first IMP dose date,
- Prior medications that continue or ends on or after the first IMP dose date.

#### *9.2.4.4.1. Medication History – Prior Medication Use*

Subjects will be queried about their prior medication use at the time points/visits indicated in the flowchart in Section 3 (see Section 8.8.2 for forbidden medications). Prior medications used by the subjects will be documented including the name of the drug, the pharmaceutical form, the strength, the dose regimen, the frequency, the route of administration, the date of initiation and discontinuation, and the reason for use.

Any medications taken in the 3 months prior to Screening Visit will be checked and recorded for subject's inclusion, in particular the use of ICS (Section 8.4).

Any medications taken in the 3 months prior to Screening Visit, including respiratory medications and especially the use of as-needed reliever medication (SABA-containing medication) and any other asthma medications, will be recorded at Screening Visit (see Section 8.8.2 for forbidden medications).

#### *9.2.4.4.2. Concomitant Medication Use*

Subjects will be queried about their concomitant medication use and their diaries checked for correct completion (Section 9.2.2.2) at the time points/visits indicated in the flowchart in Section 3 (see Section 8.8.2 for forbidden medications). Concomitant medications taken by the subjects will be documented including the name of the drug, the pharmaceutical form, the strength, the dose regimen, the frequency, the route of administration, the date of initiation and discontinuation, and the reason for use.
The reason for use or change of strength or dose regimen of a concomitant therapy may need to be reported as an AE (Section 9.2.1.1).

### 9.2.4.5. *Subsequent Therapy Counseling*

At the end of the clinical trial (Visit 4, End-of-Trial Visit) or in case of subject's withdrawal, the subsequent therapy will be at the discretion of the subject's treating physician.

# 10. TRIAL MANAGEMENT

## 10.1. Responsibilities

### 10.1.1. Investigator(s)

The Principal Investigator is accountable for the conduct of the clinical trial. Responsibilities may be delegated to appropriately qualified persons (designees).

A delegation of authority log will be filled in and signed by the Principal Investigator. In accordance with this authority log, trial site staff (e.g., sub-investigators, nurses) is authorized to perform tasks relating to the clinical trial.

### 10.1.2. Information to Investigator(s)

IMP under investigation: AQ001S 0.125 mg/ml
An Investigator's Brochure (IB) will be handed out to the Investigator before the start of the clinical trial. The IB contains all information in the Sponsor's possession necessary for the Investigator to be fully and accurately informed about the safety of AQ001S 0.125 mg/ml and the respective benefit/risk ratio.
The IB will be updated by the Sponsor at regular intervals and whenever relevant new information concerning AQ001S 0.125 mg/ml becomes available.
The Investigator will be informed about the methods for rating relevant clinical trial outcomes and for completing eCRFs.
The Investigator will be kept informed of important data that relate to the safe use of AQ001S 0.125 mg/ml as the clinical trial proceeds.

Sponsor is responsible for the ongoing safety evaluation of the IMP under investigation and will expedite the notification to the Principal Investigator and regulatory authorities of findings that are both serious and unexpected and/or that could adversely affect the safety of subjects, the conduct of the clinical trial or alter the IEC/IRB's approval to continue the clinical trial.

Comparator: Budesonide
The SmPC related to the comparator, i.e. Budesonide , will be handed out to the Investigator before the start of the clinical trial.

AxMP: Provocholine®
The technical information sheet related to the AxMP Provocholine® (safety aspects, storage information) and the guidelines of the American Thoracic Society [41] (MCh challenge test protocol) will be handed out to the Investigator before the start of the clinical trial.

Nebulizer: Pari LC Sprint nebulizer, combined with a Pari Boy Classic compressor
The IFUs of these CE-marked medical devices (Appendix 1 and Appendix 2) will be handed out to the Investigator before the start of the clinical trial.

## 10.2. Duration of the Clinical Trial

### 10.2.1. Planned Duration for an Individual Subject

For each subject, the clinical trial will last 77 to 85 days from Screening Visit, including two treatment periods of 29 (+2) days and one washout period of 14 (+2) days between treatment periods.
The duration for an individual subject may be up to a maximum of 147 days from Pre-Trial Visit if the subject is not ICS-naïve.

### 10.2.2. Time Windows Used in this Trial Including Tolerances

In this clinical trial, time windows are tolerated for the scheduling of the visits indicated in Section 3. In addition, the following time windows and tolerances apply:

| Item | Time window / tolerance |
|---|---|
| **Washout periods** | |
| Initial ICS washout period before Screening Visit | ≥ 60 days |
| Washout period between both treatment periods | 14 (+2) days |
| **Single dose 24-hour PK** | |
| Pre-dose time point | ≤ 6 hours pre-treatment |
| Post-dose time points | ± 2'; ± 2'; ± 2'; 20 ± 2'; 30 ± 2'; 45' ± 5'; 60' ± 5', 90' ± 5'; 120 ± 10'; 180' ± 15'; 240' ± 20'; 360' ± 20'; 10h ± 1h; 18h ± 2h; 24h ± 2h |
| **Abridged PK** | |
| Pre-dose time point | ≤ 6 hours prior to the last IMP administration of the treatment period |
| Post-dose time points | ± 2'; ± 2'; ± 5'; 120' ± 10'; 240' ± 20'; 360 ± 20' |
| **IMP treatment** | |
| Daily treatment time tolerance | 24 (± 2) hours between 2 doses, except:<br>- Between D1 and D2 administrations for each treatment period, where the D2 administration may occur 24 (- 4) hours after the D1 administration |
| Duration of treatment per treatment period | 29 (+2) days |

### 10.2.3. Planned duration for the clinical trial as a whole

The clinical trial is planned to last approximately 6 months, including a recruitment period of 3 months. The clinical trial will be considered completed when all subjects have completed the End-of-Trial Visit (Visit 4) except in case of unresolved safety issues.

## 10.3. Protocol Amendments

Any prospective change to the protocol will be agreed between the Principal Investigator and the Sponsor prior to its implementation.
Any such amendments will be submitted to the competent IEC/IRB and/or competent authority as required by applicable regulations.
IEC/IRB approval will, at a minimum, be requested for any change to this protocol which could affect the safety of the subjects, the objective/design of the clinical trial, any increase in dosage or duration of exposure to the IMP, an increase in the number of subjects treated, the addition of a new test or procedure, or the dropping of a test intended to monitor safety.

## 10.4. Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol or GCP requirements. The noncompliance may be either on the part of the subject, the Investigator, or the trial staff.

An Investigator must not make any changes or deviate from this protocol, except to protect the rights, safety and well-being of a trial subject in case of an emergency.
It is the responsibility of the Investigator to use continuous vigilance to identify and report deviations as soon after becoming aware of the deviation. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

All deviations from the protocol, with the reason for the deviation and the date of occurrence, must be documented and reported to the Sponsor. Sites may also be required to report deviations to the IEC/IRB, per local guidelines and government regulations.

Deviations will be reviewed and evaluated on an ongoing basis and, as necessary, appropriate corrective and/or preventive actions will be implemented by the Sponsor.

## 10.5. Withdrawal and Replacement of Subjects

### 10.5.1. Premature Subject Withdrawal

Subjects have the right to withdraw from the clinical trial at any time for any reasons, without the need to justify their decision. The Investigator also has the right to withdraw subjects in case of AEs, poor compliance, or other reasons. Since an excessive rate of withdrawals can render the clinical trial noninterpretable, any unnecessary withdrawal of subject should be avoided.

The Investigator can withdraw the subject from the clinical trial for safety reasons, including pregnancy (Section 9.2.1.1). In this case, the Investigator will immediately inform the Sponsor and will fill in an (S)AE form in the eCRF. If subject's withdrawal is due to safety reason, for example an (S)AE, the subject will be observed until complete resolution of the (S)AE. Any sequel will have to be reported. Subjects will be contacted 1 month after the complete resolution of the case before closing the subject's eCRF. Report of this contact will be recorded in the eCRF.

Investigator will withdraw the subject from the clinical trial in case of pregnancy (Section 9.2.1.1.3). In this case, the Investigator will immediately inform the Sponsor and will fill in a pregnancy notification form in the eCRF. If subject's withdrawal is due to pregnancy occurring during the clinical trial, both

mother and fetus will be observed until childbirth, upon subject's consent. The Investigator will collect all follow-up information on the outcome and send it to a Sponsor representative.

Subject's withdrawal will have to be considered by the Investigator in case of non-compliance to the clinical trial procedures, in case of smoking start and in case of use of forbidden concomitant medications. In this case, the Investigator will inform the Sponsor of this deviation to the protocol; the Sponsor will take the final decision to withdraw or not the subject from the clinical trial. If the subject is withdrawn, no new data will be collected from the trial subject, but data already collected will be cleaned before subject's eCRF closure.

For any withdrawals after clinical trial entry, i.e. from Visit 1 randomization, the Investigator will obtain all the required details and document the reason(s) for discontinuation. In addition, the Investigator will schedule, if possible, an early termination visit (Section 9.1.7).

If the reason for withdrawal of a subject is an AE, the event or laboratory test leading to the subject's withdrawal will be recorded. The Investigator will make thorough efforts to clearly document the outcome.

### 10.5.2. Subject Replacement Policy

Randomized subjects will be replaced only in case of a withdrawal proportion in excess of 15%.
Any replacement subject will be assigned to the same treatment sequence as the withdrawn subject.
Subjects withdrawn from the clinical trial for safety reasons will not be replaced.

## 10.6. Termination of the Clinical trial

If the clinical trial is terminated prematurely or suspended, the appropriate IEC/IRB and regulatory authority(ies) will be promptly informed of the termination or suspension and will be provided the reason(s) for the termination or suspension. All obligations and responsibilities of the Sponsor and the Investigator following GCP, national regulations and the Declaration of Helsinki will remain in force if the clinical trial is terminated prematurely.

### 10.6.1. Premature Termination of the Clinical Trial

Both the Investigator and the Sponsor reserve the right to terminate the clinical trial at any time. In this event, any necessary procedures will be arranged on an individual basis after review and consultation by both parties. In terminating the clinical trial, the Sponsor and the Investigator will ensure that adequate consideration is given to the protection of the subjects' interests.
Early termination of the clinical trial as a whole or by trial site may apply for the reasons detailed in the following subsections:

#### 10.6.1.1. *Early Termination of the Clinical Trial*

At any time, the clinical trial as a whole will be terminated prematurely if new toxicological or pharmacological findings or safety reports invalidate the earlier positive benefit-risk assessment.
Significant worsening of the benefit/risk ratio of AQ001S 0.125 mg/ml will lead to reevaluate the appropriateness to continue, hold or stop the clinical trial.

#### 10.6.1.2. *Early Termination of Trial Site*

At any time, the clinical trial can be terminated at the trial site if:

- The trial site cannot comply with the requirements of the protocol,

- The trial site cannot comply with GCP standards,
- The trial site cannot comply with applicable Good Manufacturing and Distribution Practice,
- The trial site's first subject is not recruited within 2 weeks after initiation of the trial site,
- The required recruitment rate is not met.

Should the clinical trial be prematurely terminated, all trial materials (completed, partially completed, and blank eCRFs, IMPs, etc.) must be returned to the Sponsor.

## 10.7. DATA HANDLING AND RECORD KEEPING

### 10.7.1. Source Data and Records

Source data are defined as all information related to clinical findings, observations, or other activities in the clinical trial, written down in original records or certified copies of original records, allowing reconstruction and evaluation of the clinical trial.
The Investigator will maintain adequate source records (e.g., case histories or subject files for each subject enrolled). Source records should be preserved for the maximum period of time required by local regulations.
For each subject enrolled, the Investigator will indicate in the source record(s) that the subject participates in this clinical trial.
All data entered in the eCRF must be supported by source data in the subject records.
The Investigator will permit trial-related monitoring, audit(s), IEC/IRB review(s), and regulatory inspection(s), by providing direct access to the source data records.
The Investigator may authorize site staff (e.g., sub-Investigators, nurses) to enter clinical trial data into the eCRF. This must be documented in the delegation of authority log signed by the Investigator.

### 10.7.2. Case Report Forms

For each subject enrolled, an electronic case report form (eCRF) will be completed within the electronic data capture (EDC) system and approved by the Investigator or an authorized sub-Investigator.

Trial site staff (e.g., study nurse) will be responsible for entering subject data into the validated EDC system. All site staff will be trained on the EDC system and trial specific eCRFs prior to receiving access to the live database for data entry.

The site is also provided with the approved eCRF completion guidelines which will assist in data entry and data issues/questions. The site will be notified once the database is active to begin data entry. Additional site training may be provided as refreshers throughout the clinical trial, if needed. All people allowed to enter or change eCRF data must be listed in the delegation of authority log.

Changes to Case Report Form Data
Monitors will perform source data verification (SDV) as defined for the clinical trial.
If any errors or discrepancies in the eCRFs are found during data entry or review, discrepancies will be generated automatically within the EDC system, and 'manual' queries will be created by either a monitor or Data Management.
Discrepancies and queries can only be corrected by the Investigator(s) or other authorized site staff. An audit trail will document all changes to the data over the entire clinical trial period. If the reason for a change is not obvious, a comment must be supplied in the query's response, stating the reason for the change, prior to closing. The clinical trial monitor should provide guidance to Investigator(s) and the Investigator(s)' designated representatives on making such corrections.

Once queries have been resolved by the site staff, the resolutions are assessed by Data Management. If the query response provided confirms the data as correct, the discrepancy will be closed. If the response does not adequately address the question raised, a new query will be issued for further clarification.
Manual checks are performed, and programs are run throughout the clinical trial until the data is clean and the database is ready for lock. All discrepancies will be resolved prior to database lock. There will be a final run of the programmed checks to ensure all discrepancies are closed out, SDV will be confirmed as complete by the monitor, and all eCRFs will be approved by the Investigator prior to database lock.

### 10.7.3. Investigator's Site File

The Investigator is responsible for maintaining all records to enable the conduct of the clinical trial to be fully documented. Essential documents as required by GCP guidelines and regulations (e.g., copies of the protocol, trial approval letters, all original informed consent forms, site copies of all eCRFs, IMP dispensing and accountability logs, correspondence pertaining to the clinical trial, etc.) should be filed accurately and kept by the Investigator for the maximum period of time required by local regulations.
The Investigator is responsible for maintaining a confidential subject identification code list, which provides the unique link between named source records and eCRF data for the Sponsor. The Investigator must arrange for the retention of this confidential list for the maximum period of time required by local regulations.
No clinical trial document should be destroyed without prior written agreement between the Investigator and the Sponsor. Should the Investigator elect to assign the trial documents to another party, or move them to another location, the Sponsor must be notified in writing and will have to give its formal written agreement prior to implementing such change.

### 10.7.4. Provision of Additional Information

On request, the Investigator will supply the Sponsor with additional data relating to the clinical trial, or copies of relevant source records, ensuring that the subject's confidentiality is maintained. In case of particular issues or governmental queries, it is also necessary to have access to the entire clinical trial records, provided that the subject's confidentiality is protected in accordance with applicable regulations.

### 10.7.5. Safety Monitoring

The Sponsor Pharmacovigilance Manager, the Safety Monitor and the Investigator will review relevant data periodically during the clinical trial and will give advice on the continuation, modification, or termination of the clinical trial. The benefit/risk of AQ001S 0.125 mg/ml (Section 6.3.1 and described in more details in the AQ001S' IB) will be reevaluated by the Sponsor Pharmacovigilance Manager at each SAE report. His/her evaluation and clinical justification will be entered in the eCRF in a text section of the AE reporting form. In case his/her evaluation leads to a modification of the previous benefit/risk, the Sponsor and Investigator will decide together of the corrective actions that need to be implemented.
An independent data monitoring committee will not be established by the Sponsor for the clinical trial.
A written clinical trial-specific safety plan will define in detail the responsibilities and procedures used for recording, reporting and assessing AE and any safety information during the clinical trial conduct.

# 11. STATISTICAL METHODS AND SAMPLE SIZE

## 11.1. Determination of Sample Size

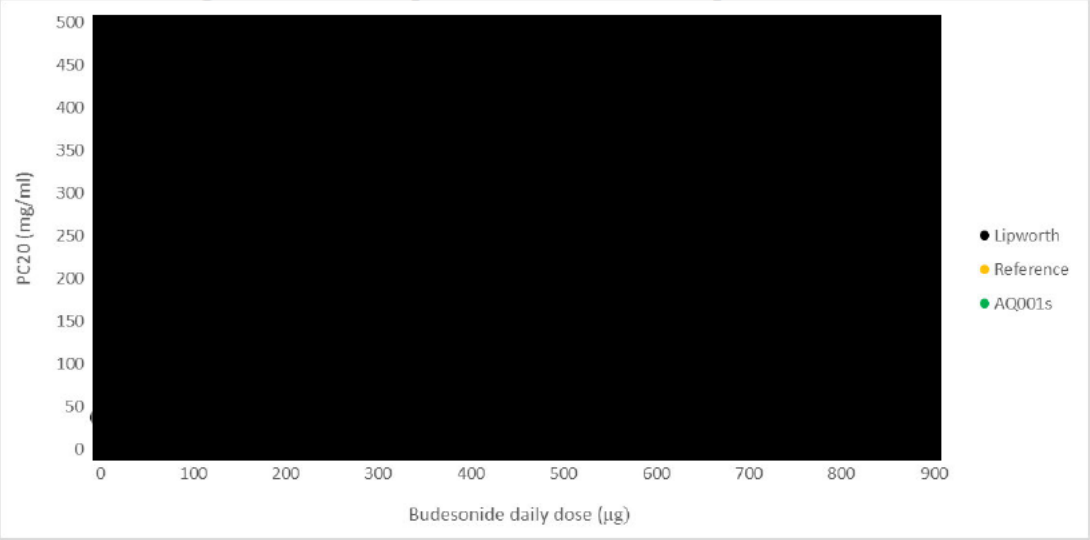
A total of 24 subjects will be recruited into the clinical trial. Considering potential dropouts, a total of 20 subjects are anticipated to complete the clinical trial.

As the PC20 is the primary endpoint, the sample size was calculated based on the work of Lipworth *et al*. [44] and of Kraan *et al*. [55]. Both authors studied the response of patients in terms of PC20 to various doses of inhaled budesonide, as described in the 2 calculation options below.

### 11.1.1. Calculation Option 1 (Lipworth)

Nonclinical studies conducted by Aquilon in several mouse models of asthma showed that AQ001S is times as as budesonide suspension ( ) [28,37].
In the study published by Lipworth *et al*. (2005) [44], analysis of the PC20 (mg/ml) means and standard deviation (SD) data for each budesonide dose ( , and mg budesonide/day) showed that the dose-response is linear between mg budesonide for the means (SD constants were taken as the maximal SD observed in measurements in G*Power) (Figure 4, black dots).

**Figure 4 - Dose-response curve based on Lipworth *et al*. 2005**




Based on that, the response of the comparator was calculated for a dose of 0.125 mg budesonide (budesonide daily dose in BOREAS) (Figure 4, Reference, yellow dot). The test response for AQ001S 0.125 mg/ml in BOREAS was then calculated using an fold ( ) (Figure 4, green dot) and a -fold (see Aquilon nonclinical data above [28,37]) higher dose than the comparator.
The -fold factor was selected arbitrarily and is considered as , given that any factor below -fold would not show a sufficient difference in superiority between AQ001S and the comparator.

A within-subject test-retest correlation was then calculated using the PC20 data from Kraan *et al*. (1988) [55] and the worst observation correlation, i.e. the correlation between PC20 values from week 2 to week 4 in subjects treated with 0.2 mg budesonide, was selected for the sample size calculation.The sample size was calculated using the software G*Power, based on the following assumptions:

- t-test; Means: Difference between two dependent means (matched pairs),
- 1 tailed (only superior),
- alpha error probability: 5% (0.05),
- power (1-beta probability): 90% (0.9) or 80% (0.8).

### 11.1.2. Calculation Option 2 (Kraan)

Nonclinical studies conducted by Aquilon in several mouse models of asthma showed that AQ001S is times as as budesonide suspension ( ) [28,37].

In the study published by Kraan *et al*. [55], the effect of a dose of mg budesonide/day tested in the study on the PC20 (mg/ml) was assumed to be at a plateau [39] and similar to the effect of a dose of mg budesonide/day (Figure 5, black dot at µg). Analysis of the mean difference in PC20 (mg/ml) at 4 weeks for the doses of mg budesonide and mg budesonide/day showed that the

for the means and the SD (Figure 5 and Figure 6, black dots).

**Figure 5 - Dose-response curve (means) based on Kraan *et al*. 1988**

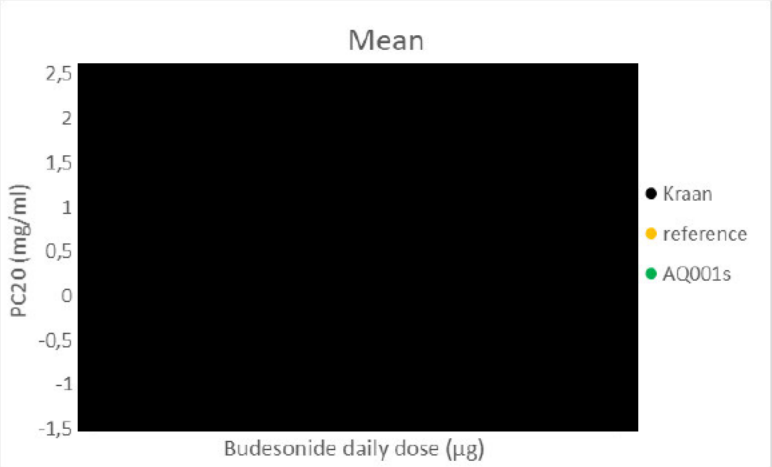
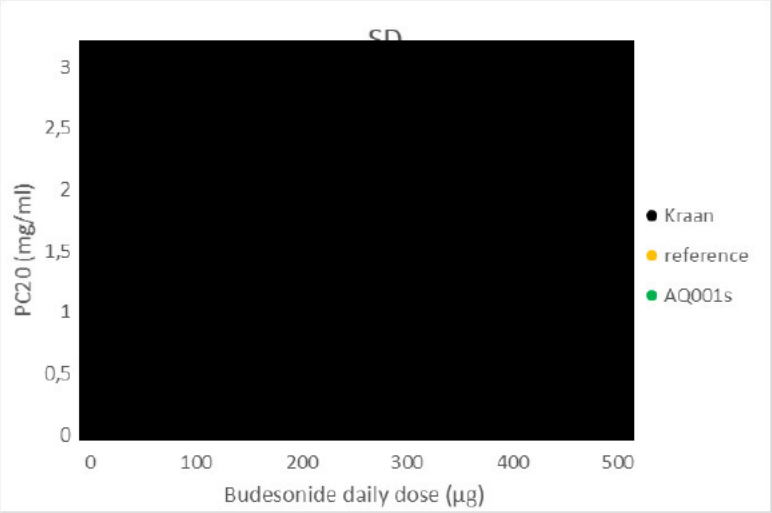



**Figure 6 - Dose-response curve (SD) based on Kraan *et al*. 1988**




Based on that, the response of the comparator was calculated for a dose of 0.125 mg budesonide (budesonide daily dose in BOREAS) (Figure 5 and Figure 6, Reference, yellow dot). The test response for AQ001S 0.125 mg/ml in BOREAS was then calculated using a fold ( ) (Figure 5 and Figure 6, green dot) and a fold (see Aquilon nonclinical data above [28,37]) higher dose than the comparator.

The -fold factor was selected arbitrarily and is considered as the , given that any factor below -fold would not show a sufficient difference in superiority between AQ001S and the comparator.

A within-patient test-retest correlation was then calculated using the PC20 data from Kraan *et al.* (1988) [55] and the worst observation correlation, i.e. the correlation between PC20 values from week 2 to week 4 in patients treated with mg budesonide, was selected for the sample size calculation.

The sample size was calculated using the software G*Power, based on the following assumptions:

- t-test; Means: Difference between two dependent means (matched pairs),
- 1 tailed (only superior),
- alpha error probability: 5% (0.05),
- power (1-beta probability): 90% (0.9) or 80% (0.8).

### 11.1.3. Sample Size Results

The sample sizes obtained according to the calculation options 1 and 2 and using a power (1-beta probability) of 80% (0.8) are given in Table 3.

**Table 3 – Calculated sample sizes based on the PC20 as a primary endpoint**

| (power=0.8) | **Required sample size - number of subjects completing the trial to show superiority of AQ001S over comparator in terms of budesonide dose reduction of:** | |
|---|---|---|
| | **-fold** | **-fold** |
| Lipworth (option 1) | | |
| Kraan (option 2) | | |

Calculations based on the dose-response observed by Lipworth et al. [44] (option 1) or by Kraan et al. [55] (option 2)

The sample size calculations based on the PC20 as a primary endpoint in BOREAS indicate that a sample size of 20 subjects completing the clinical trial is enough to demonstrate an effect of AQ001S 0.125 mg/ml over the comparator with a -fold factor (arbitrary factor, worst case) as well as with a -fold factor between AQ001S 0.125 mg/ml and the comparator with a power of 80%.

## 11.2. Randomization, Stratification and Code Release

The allocation of the patients to the treatment sequences A and B (Section 8.9) is done by 1 to 1 block randomization.
The randomization list will be generated using a pseudo random number generator and a seed number that ensures that the allocation is non-predictable and reproducible. The randomization list will contain a mirror list to allow for subject replacements.
As this is an open-label clinical trial, no blinding is done.

## 11.3. Statistical Analysis

A formal statistical analysis plan (SAP) describing all details of the analyses to be performed will be prepared by the clinical trial statistician and approved by the Sponsor prior to the start of the statistical analysis.

Statistical analyses will be performed using SAS® version 9.4 or later. Statistical tests and confidence intervals will be two-sided at 5% significance level.

### 11.3.1. Populations for Analysis

Efficacy (EFF) population
The EFF population encompasses all subjects that completed both treatment periods and for which the primary efficacy parameter PC20 is available at baseline (Visit 1) and after both treatment periods (Visit 2 and Visit 4).
The EFF population is a per-protocol set, in the sense that it is a subset of the full analysis set, where subjects with major protocol deviations which may have an impact on the evaluation of the primary clinical trial outcome parameter will be excluded.
Examples of major and minor protocol deviations will be described in the SAP.
A final decision about the classification of protocol deviations and their consequences regarding assignment of subjects to analysis sets will be made during the data review meeting. Decisions and outcome will be approved by the Sponsor.

The safety (SAF) population will include all randomized subjects who received at least one dose of IMP (AQ001S 0.125 mg/ml or comparator).

Pharmacodynamics (PK) population
The PK population includes all subjects, which have two full evaluable PK profiles and did not have protocol deviations relevant to the evaluation of PK.

### 11.3.2. Primary Efficacy Endpoint and Analysis Plan

The primary efficacy analysis will be evaluated on the EFF population. The primary efficacy endpoint will be the change from baseline in PC20 after each treatment period (baseline is defined at Visit 1).

For primary efficacy analysis, the mean PC20 changes from baseline between AQ001S 0.125 mg/ml and the comparator will be compared by analysis of covariance (ANCOVA) for crossover design. A p-value will be calculated for the difference of the parameter between AQ001S 0.125 mg/ml and comparator. If this p-value is significant and the difference prefers AQ001S 0.125 mg/ml, superiority is shown.
Additionally, an adjusted 95%-confidence interval for the mean difference between AQ001S 0.125 mg/ml and the comparator will be obtained by the ANCOVA.

### 11.3.3. Safety Endpoints and Analysis Plan

The safety will be evaluated collecting the following information:

- AEs, SAEs, including asthma exacerbations,
- General tolerability: vital signs, ECG, physical examination,
- Laboratory parameters: hematology, biochemistry and urinalysis,
- Local tolerability:
  - Increased bronchial irritability,
  - Paradoxical bronchospasm,
  - Oropharyngeal examination (e.g. vocal cord myopathy, fungal infection),
- $FEV_1$ and FVC, measured by spirometry,
- HPA axis function: assessment of urinary cortisol/creatinine ratio in first waking urine.

The safety endpoints will be analyzed for the SAF population. Safety analyses will be descriptive in nature and no hypothesis testing is planned.

The treatment groups will be compared in a descriptive way. Tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics (at least number of observations, mean, standard deviation, median, minimum and maximum) will be used to analyze continuous (quantitative) data.

Measurements (such as ECG, vital signs, or laboratory parameters) or AEs will be assigned to treatments based on the actual treatment at the planned time of the measurement or on the recorded time of AE onset (concept of treatment emergent AEs).

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). Frequency, severity and causal relationship of AEs will be tabulated by treatment, system organ class and preferred term. Absolute and relative frequencies of subjects with at least one AE will be presented and the total number of AEs will be given by treatment group. SAEs and other significant AEs will be evaluated separately.

### 11.3.4. Pharmacokinetic Endpoints and Analysis Plan

The PK endpoint which is the PK profile of budesonide in plasma will be evaluated through the PK parameters listed in Section 9.2.3.4, calculated for each treatment period.

The PK analysis will be performed based upon the PK set. The primary focus of the statistical analysis of this section is to describe and characterize the PK of AQ001S 0.125 mg/ml and the comparator. Thus, there are no formal hypotheses being tested.

The individual plasma concentrations of budesonide will be listed and tabulated by treatment and time point.
Descriptive statistics for the PK parameters will be calculated by treatment. The tables will include at least the number of observations, mean, standard deviation, median, minimum and maximum, and additionally the geometric mean and the geometric coefficient of variation (gCV).

### 11.3.5. Secondary Efficacy/Pharmacodynamic Endpoints and Analysis Plan

The secondary efficacy/PD endpoints will be the changes from baseline, after each treatment period, in:

- Symptom scores,
- Use of as-needed reliever medications,
- FeNO,
- Blood eosinophil count,
- $FEV_1$,
- FIVC.

The secondary efficacy and PD analysis will be performed on the SAF population. Those parameters will also be evaluated descriptively. Therefore, tabulations of frequencies/proportions will be used for the evaluation of categorical (qualitative) data, and tabulations of descriptive statistics (at least number of observations, mean, standard deviation, median, minimum and maximum) will be used to analyze continuous (quantitative) data.

### 11.3.6. Handling of Data and Specific Analyses

#### 11.3.6.1. *Handling of Dropouts or Missing Data*

In general, missing data will not be imputed. Calculations pertaining to person-year computations will be based on observed values only. Only in case of missing body weight will the last available weight measurement be used for calculating the dose per kg bodyweight (last observation carried forward).

#### 11.3.6.2. *Adjustments for Covariates*

The ANCOVA models used for primary analysis will be adjusted for the baseline value, treatment sequence, period and within-subject sequence.

#### 11.3.6.3. *Active Control Clinical Trials Intended to Show Non-Inferiority*

Not applicable.

#### 11.3.6.4. *Data Presentation*

Individual response data and other relevant clinical trial information will be presented in tables, with supporting individual data listings.

#### 11.3.6.5. *Interim Analysis*

No interim analysis will be performed in the framework of this clinical trial. AQ001S 0.125 mg/ml's benefit/risk ratio will be evaluated on an ongoing basis by the Sponsor's Pharmacovigilance Manager. Any modification of the benefit/risk ratio will be immediately communicated by the Sponsor's Pharmacovigilance Manager to the Investigator.

# 12. ETHICAL/REGULATORY LEGAL AND ADMINISTRATIVE ASPECTS

## 12.1. Ethical/Regulatory Framework

This clinical trial will be conducted in accordance with the ethical principles laid down in the Declaration of Helsinki. The clinical trial protocol and any subsequent amendment(s) will be submitted to an Independent Ethics Committee (IEC)/Institutional Review Board (IRB) and to the Regulatory Authority. The clinical trial will be conducted in compliance with the protocol, GCP guidelines, and applicable regulatory requirements.

The regulatory application or submission for regulatory approval will be made by the Sponsor or designated third party (e.g., a contract research organization [CRO]) as required by national law.

## 12.2. Approval of Clinical Trial Documents

The clinical trial protocol, a sample of the subject information and informed consent form, any other materials provided to the subjects, and further requested information will be submitted by the Sponsor or the Investigator to the appropriate IEC/IRB and the Regulatory Authority. The clinical trial must be approved by the IEC/IRB and the Regulatory Authority before any IMP may be shipped to the trial sites and before any subject is exposed to a trial related procedure.

The Sponsor, the Investigator, and any third party (e.g., CRO) involved in obtaining approval must inform each other in writing that all ethical and legal requirements have been met before the first subject is enrolled in the clinical trial.

## 12.3. Subject Information and Informed Consent

The Investigator will obtain freely given written consent from each subject after an appropriate explanation of the aims, methods, anticipated benefits, potential hazards, and any other aspect of the clinical trial which is relevant to the subject's decision to participate.

The informed consent form must be signed, with name, date and time noted by the subject, before the subject is exposed to any trial-related procedure, including screening tests for eligibility.

The Investigator will explain that the subjects are completely free to refuse to enter the clinical trial or to withdraw from it at any time, without any consequences for their further care and without the need to justify. The Investigator will fill in the informed consent section of the eCRF for each subject enrolled. Each subject will be informed that his/her medical (source) records may be reviewed by the clinical trial monitor, a quality assurance auditor, or a health authority inspector, in accordance with applicable regulations, and that these persons are bound by confidentiality obligations.

### 12.3.1. Subject's Data Protection

Besides the subject's agreement to participate in the clinical trial, the informed consent form will also give to the subject detailed information on the intended treatment of his/her data. The EU GDPR is indeed applicable to any clinical trial. The subject's consent will include both the consent to participate in the clinical trial and his/her explicit consent to the intended treatment of his/her data.

In consequence, the Sponsor has the obligation to implement the appropriate technical and organizational measures to ensure and be able to demonstrate that the personal data are processed in accordance with the data protection rules (Article 24 of GDPR). The Sponsor will ensure compliance to all the data protection rules in GDPR, including ensuring respect of the data protection principles, appointing a responsible person for Data Protection, maintaining records of processing activities and facilitating the exercise of individuals' rights. The name and contact details of the responsible person for Data Protection will be mentioned in the informed consent form.

In addition, the subjects will be informed on the purposes of the processing as well as the legal bases of this data treatment (legal obligation of the sponsor to report safety & efficacy data to the competent authorities [Article 6(1)(c) of GDPR in conjunction with Article 9(2)(i)] and explicit consent of the subject [Article 6(1)(a) of GDPR in conjunction with Article 9(2)(a)] [56,57]), on the identity of the controller (sponsor), its contact details and, where applicable, those of the controller's representative; the recipients or categories of recipients of the personal data, if any; the finalities of the treatment of his/her data, the storage period (or if not possible, criteria used to determine that period), the clinical research purposes, the fair processing of his/her data, and on his/her data subjects rights (access; rectification; erasure; restriction on processing; objection to processing and data portability) as required by the EU GDPR. The subjects will be informed of their right to lodge a complaint with a supervisory authority. Subjects will also be informed that their data could be transferred to another party in another country. In case of such data transfer, the Sponsor will maintain high standards of confidentiality and protection of subject personal data and ensure that any data processor used is reliable and can make sufficient data protection guarantees (implementing appropriate technical and organizational measures). The Sponsor will establish Standard Contractual Clauses (SCC) on data transfer ensuring that all parties working with the sponsor are committed to protecting and keeping the subject's personal data confidential. The subjects will also be informed that the treatment of his/her data will not give him/her any rights for any other compensation than the one linked to their participation to the clinical trial.

Finally, as well as for GCP compliance, GDPR compliance is, in Belgium, in the framework of a clinical trial, the competence of the consulted IEC/IRB. The responsibility for the GDPR compliance check of the clinical trial dossier lies with the sponsor.

## 12.4. Confidentiality of Subject Data

The Investigator will ensure that the subject's confidentiality is preserved. Per GCP, on eCRFs or any other documents submitted to the Sponsor, the subjects will not be identified by their names, but by a unique subject identifier (data pseudonymization) (Section 8.4.5). Documents not intended for

submission to the Sponsor, i.e., the confidential subject identification code list, original consent forms, and source records, will be maintained by the Investigator in strict confidence.

# 13. QUALITY CONTROL AND QUALITY ASSURANCE

## 13.1. Periodic Monitoring

The monitor will contact and visit the Investigator periodically to review all trial-related source data/records, verify the adherence to the protocol and the completeness, correctness and accuracy of all eCRF entries compared to source data. The Investigator will co-operate with the monitor to ensure that any discrepancies identified are resolved.

A monitoring plan will be approved by the Sponsor before trial site initiation.

The monitor must be given direct access to source documents (original documents, data and records). Direct access includes permission to examine, analyze, verify, and reproduce any records and reports that are important to the evaluation of the clinical trial. Source data will be available for all data in the eCRFs, including all laboratory results.

## 13.2. Audit and Inspection

The Investigator will make all trial-related source data and records available to a qualified quality assurance auditor mandated by the Sponsor, after reasonable notice, or to IEC/IRB/regulatory inspectors. The main purpose of an audit or inspection is to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for the assessment of safety and efficacy of AQ001S 0.125 mg/ml have been reported to the Sponsor.

# 14. REPORTING AND PUBLICATION

## 14.1. Clinical Study Report

A clinical study report (in accordance with relevant guidelines and the Sponsor's procedures) will be prepared by the Sponsor after completion of the clinical trial. The Principal Investigator will approve the final clinical study report after review.

## 14.2. Publication Policy

The results of this clinical trial will be shared in the European Union Clinical Trials Register and in other mandatory registers as appropriate and according to the regulatory requirements.
The results of this clinical trial may be published or presented at scientific meetings.
If this is envisaged by the Investigator, the Investigator agrees to inform the Sponsor and to submit for review and approval all manuscripts or abstracts to the Sponsor prior to submission to an editorial board or scientific review committee. This will allow the Sponsor to protect proprietary information and to provide comments based on information that may not yet be available to the Investigator.
Authorship will be determined by mutual agreement.
The publication and disclosure policies are addressed in a separate agreement with the trial site.

# 15. LIABILITIES AND INSURANCE

In order to cover any potential damage or injury occurring to a subject in association with the IMPs or participation in the clinical trial, the Sponsor will contract insurance in accordance with local regulations.
The Investigator is responsible for dispensing the IMPs according to this protocol and for the secure storage and safe handling of the IMPs throughout the clinical trial.
The financing and insurance details are addressed in a separate agreement with the trial site.

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# 17. APPENDICES

## APPENDIX 1: PARI LC SPRINT NEBULIZER – INSTRUCTIONS FOR USE

See Appended document.

## APPENDIX 2: PARI BOY CLASSIC COMPRESSOR – INSTRUCTIONS FOR USE

See Appended document.

## APPENDIX 3: LABORATORY TESTS (INCLUDING OPTIONAL URINALYSIS AND URINARY CORTISOL/CREATININE RATIO)

| Medium | Variable | Data type | Value range (Female) | Value range (Male) | Units |
|---|---|---|---|---|---|
| **BLOOD HEMATOLOGY AND BIOCHEMISTRY** | | | | | |
| Blood | Hemoglobin | number | | | g/dL |
| Blood | Hematocrit | number | | | % |
| Blood | Red Blood Cell count | number | | | $10^6$/microL |
| Blood | Mean Corpuscle Hemoglobin (MCH) | number | | | picog |
| Blood | Mean Corpuscle Volume (MCV) | number | | | femtoL |
| Blood | Reticulocyte count | number | | | $10^3$/microL |
| Blood | White Blood Cell count | number | | | $10^3$/microL |
| Blood | Neutrophils | number | | | $10^3$/microL |
| Blood | Lymphocytes | number | | | $10^3$/microL |
| Blood | Monocytes | number | | | $10^3$/microL |
| Blood | Eosinophils | number | | | $10^3$/microL |
| Blood | Basophils | number | | | $10^3$/microL |
| Blood | Neutrophils | number | | | % |
| Blood | Lymphocytes | number | | | % |
| Blood | Monocytes | number | | | % |
| Blood | Eosinophils | number | | | % |
| Blood | Basophils | number | | | % |
| Blood | Platelet count | number | | | $10^3$/microL |
| Blood | Urea | number | | | mg/dL |
| Blood | Creatinine | number | | | mg/dL |
| Blood | Uric acid | number | | | mg/dL |
| Blood | Bicarbonate | number | | | mmol/L |
| Blood | Sodium | number | | | mmol/L |
| Blood | Potassium | number | | | mmol/L |
| Blood | Chloride | number | | | mmol/L |
| Blood | Calcium | number | | | mmol/L |
| Blood | Phosphorus | number | | | mg/dL |
| Blood | Magnesium | number | | | mg/dL |
| Blood | Fasting glucose | number | | | mg/dL |
| Blood | non-fasting glucose | number | | | mg/dL |
| Blood | Hemoglobin (Hb) A1c | number | | | mmol/mol |
| Blood | Amylase | number | | | U/L |
| Blood | Lipase | number | | | U/L |

| Medium | Variable | Data type | Value range (Female) | Value range (Male) | Units |
|---|---|---|---|---|---|
| Blood | Triglycerides | number | | | mg/dL |
| Blood | Total Cholesterol | number | | | mg/dL |
| Blood | High density lipoprotein (HDL) Cholesterol | number | | | mg/dL |
| Blood | Low density lipoprotein (LDL) Cholesterol | number | | | mg/dL |
| Blood | Cortisol | number | | | µg/dL |
| Blood | Serum glutamic oxaloacetic transaminase (SGOT) | number | | | IU/L |
| Blood | Serum glutamic pyruvic transaminase (SGPT) | number | | | IU/L |
| Blood | Gamma-glutamyl transferase (GGT) | number | | | IU/L |
| Blood | Alkaline Phosphatase | number | | | IU/L |
| Blood | Total Bilirubin | number | | | mg/dL |
| Blood | Direct Bilirubin | number | | | mg/dL |
| Blood | Total protein | number | | | g/dL |
| Blood | Albumin | number | | | g/dL |
| Blood | Lactate dehydrogenase (LDH) | number | | | U/L |
| Blood | C-reactive protein | number | | | mg/L |
| Blood | Human chorionic gonadotropin (hCG) | number | | | IU/L |
| **OPTIONAL URINALYSIS** | | | | | |
| Urine | Nitrites | positive/negative | | | None |
| Urine | Urine gravity | number | | | None |
| Urine | pH | number | | | None |
| Urine | White Blood Cell count | number | | | None |
| Urine | Leucocyte Esterase | positive/negative | | | None |
| Urine | Protein | positive/negative | | | None |
| Urine | Glucose | positive/negative | | | None |
| Urine | Ketones | positive/negative | | | None |
| Urine | Bilirubin | positive/negative | | | None |
| Urine | Hemoglobin | positive/negative | | | None |
| Urine | Red Blood Cell count | number | | | #/microL |

[26] Limit = acceptable range if outside normal value, as appreciated by the Investigator (clinically significant or not)

| Medium | Variable | Data type | Value range (Female) | Value range (Male) | Units |
|---|---|---|---|---|---|
| Urine | Urine casts | number | | | #/microL |
| Urine | Leukocytes | number | | | #/microL |
| **URINARY CORTISOL/CREATININE RATIO (as established by )** | | | | | |
| Urine | Cortisol/creatinine ratio | number | | | microg cortisol/g creatinine |

## 18. PROTOCOL SIGNATURES

# Signature of the Sponsor's Representatives

This clinical trial is intended to be conducted in compliance with the protocol, Good Clinical Practice and applicable regulatory requirements.

| | | _____/_____/_____ |
|---|---|---|
| **Chief Executive Officer on behalf of the Sponsor** | **Signature** | **Date (DD/MMM/YYYY)** |

| | | _____/_____/_____ |
|---|---|---|
| **Clinical and Project Leader on behalf of the Sponsor** | **Signature** | **Date (DD/MMM/YYYY)** |

# Signature of the Principal Investigator

I have read the protocol carefully and hereby confirm that it is scientifically and ethically sound. I agree to implement and conduct this clinical trial diligently, in strict compliance with the protocol, ICH (step 5) 'Guidance on Good Clinical Practice [E6]' and all applicable laws and regulations.

_____/_____/_____

**Principal Investigator** **Signature** **Date (DD/MMM/YYYY)**

# Signature of the Biostatistician

This clinical trial is intended to be conducted in compliance with the protocol, Good Clinical Practice and applicable regulatory requirements.

| | | |
|---|---|---|
| | | _____/_____/_____ |
| **Biostatistician** | **Signature** | **Date (DD/MMM/YYYY)** |

# 19. DOCUMENT HISTORY

| VERSION | HISTORY | ISSUE DATE |
|---|---|---|
| 1.0 | Creation | 28/FEB/2020 |
| 2.0 | Review after IEC feedback (Comité d'Ethique Hospitalo-Facultaire Universitaire de Liège) dated 24 March 2020:<br>- Changes in title<br>-<br>- Emphasis on the primary efficacy parameter, e.g. PC20, explaining the need for this assessment at Visits 1 (baseline), Visit 2 (end of first treatment period) and Visit 4 (end of second treatment period)<br>- Add of minimum BMI as inclusion criterion (18.5 kg/m2)<br>- Amendment of maximum BMI from 30 to 29 kg/m2 as inclusion criterion<br>- Addition of HIV and COVID-19 infections as exclusion criterion<br>- Add of assessment of COVID-19 symptoms in physical and clinical examination<br>- Ad of timepoint in PK blood collection<br>- Clarifications where needed<br>- Change of compressor for the nebulizer used: Pari Boy Classic, which is compliant with the new Medical Device Regulation, instead of Pari TurboBoy SX | 21/AUG/2020 |

| | | |
|---|---|---|
| 3.0 | Review before clinical trial start:<br>- Estimated clinical start postponed to June 2021<br>- Addition of the sponsor's medical advisor for the trial contacts<br>- Amendment to the name of the company doing the packaging and labeling of IMPs<br>- Change of Bioanalysis Laboratory<br>- Clarification in the protocol of MCh challenge test to ensure subject's safety during the test (PC20 endpoint)<br>- Amendment of email address for AEs reporting to be in line with Safety Management Plan<br>- Other clarifications where needed | 15/JUN/2021 |