Imperial College London

Effect of the CRTH2 antagonist OC459 on the Response to Rhinovirus Challenge in Asthma

Clinical Trial Protocol
Version 6.0, 4th July 2017

Main Sponsor:	Imperial College London
Funder:	Medial Research Council
Study Coordination Centre:	Airways Disease Infection Section, National Heart & Lung Institute (NHLI), St Mary's Campus, Imperial College London
REC reference:	15/LO/1666
EudraCT reference:	2015-002555-10

Protocol authorised by:

Name & Role	**Date**	**Signature**
Prof Sebastian Johnston	4th July 2017	

Study Management Group

Chief Investigator:	Professor Sebastian Johnston
Co-investigators:	Dr Hugo Farne Dr David Jackson Dr Ross Walton
Statistician:	Nicholas Johnson and Professor Toby Prevost, Imperial Clinical Trials Unit statistics team
Trial Management:	This will be the responsibility of the Chief Investigator and Co-Investigators.

Study Coordination Centre

For general queries, supply of trial documentation, and collection of data, please contact:

Study Coordinator:	Dr Hugo Farne	
Address:	Airways Disease Infection Section, National Heart & Lung Institute, Wright Fleming Building, St Mary's Campus, Imperial College, Norfolk Place, W2 1PG, London	
Tel:	020 7594 3751	E-mail: h.farne@imperial.ac.uk
Fax:	020 7262 8913	

Clinical Queries

Clinical queries should be directed to Dr Hugo Farne, who will direct the query to the appropriate person.

Sponsor

Imperial College London is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Clinical Trials Manager at:

Joint Research Compliance Office
5L10C, 5th Floor Lab Block
Charing Cross Hospital
Fulham Palace Road
London, W6 8RF
Tel: 0203 311 0204
Fax: 0203 311 0203

Funder

This study is funded by a Medical Research Council (MRC) Biomedical Catalyst: Developmental Pathway Funding Scheme (DPFS) grant. Atopix Therapeutics Ltd are providing the Investigational Medicinal Product (IMP), OC459, and placebo free of charge in sufficient quantities for the purpose of the trial.

This protocol describes the Effect of the CRTH2 Antagonist OC459 on the Response to Rhinovirus Challenge in Asthma (ORCA) study and provides information about procedures for entering participants. The protocol should not be used as a guide for the treatment of other participants; every care was taken in its drafting, but corrections or amendments may be necessary. These will be circulated to investigators in the study, but centres entering participants for the first time are advised to contact the trials centre to confirm they have the most recent version.
Problems relating to this trial should be referred, in the first instance, to the study coordination centre.
This trial will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and the International Conference on Harmonisation Good Clinical Practice (ICH GCP) guidelines. It will be conducted in compliance with the protocol, the Data Protection Act and other regulatory requirements as appropriate.

Table of Contents

GLOSSARY OF ABBREVIATIONS

ACQ	Asthma Control Questionnaire
AE	Adverse Event
API	Active Pharmaceutical Ingredient
AQLQ	Asthma Quality of Life Questionnaire
AR	Adverse Reaction
ATS	American Thoracic Society
BAL	Bronchoalveolar Lavage
BEC	Bronchial Epithelial Cell
BTS	British Thoracic Society
CRF	Case Report Form
CRP	C-Reactive Protein
CRTH2	Chemoattractant Receptor-Homologous molecule expressed on Th2 cells
DMP	Data Management Plan
ECG	Electrocardiogram
EDTA	EthyleneDiamine Tetraacetic Acid
ELF	Epithelial Lining Fluid
ELISA	Enzyme-Linked ImmunoSorbent Assay
FACS	Fluorescence-Activated Cell Sorting
FeNO	Fraction of exhaled Nitric Oxide (NO)
FEV_1	Forced Expiratory Volume in One Second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
ICS	Inhaled Corticosteroid
IgE	Immunoglobulin E
IL	Interleukin
IMP	Investigational Medicinal Product
IUD	Intrauterine device
IUS	Intrauterine system
JRCO	Joint Research Compliance Office
LABA	Long Acting Beta Agonist
LRT	Lower Respiratory Tract
MDI	Metered Dose Inhaler
MHRA	Medicines and Healthcare Products Regulatory Agency
mRNA	Messenger Ribonucleic Acid (RNA)
MSD	Meso Scale Discovery [a multiplex immunoassay]
PC_{20}	Provocation concentration [of histamine] producing a 20% fall in FEV_1
PEF	Peak Expiratory Flow
PGD_2	Prostanglandin D_2
PHA	Phytoheamagglutinin
PPB	Parts Per Billion
qPCR	Quantitative Polymerase Chain Reaction (PCR)
REC	Research Ethics Committee
RV	Rhinovirus
RV-16	Rhinovirus serotype 16
SABA	Short Acting Beta Agonist
SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrix
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction

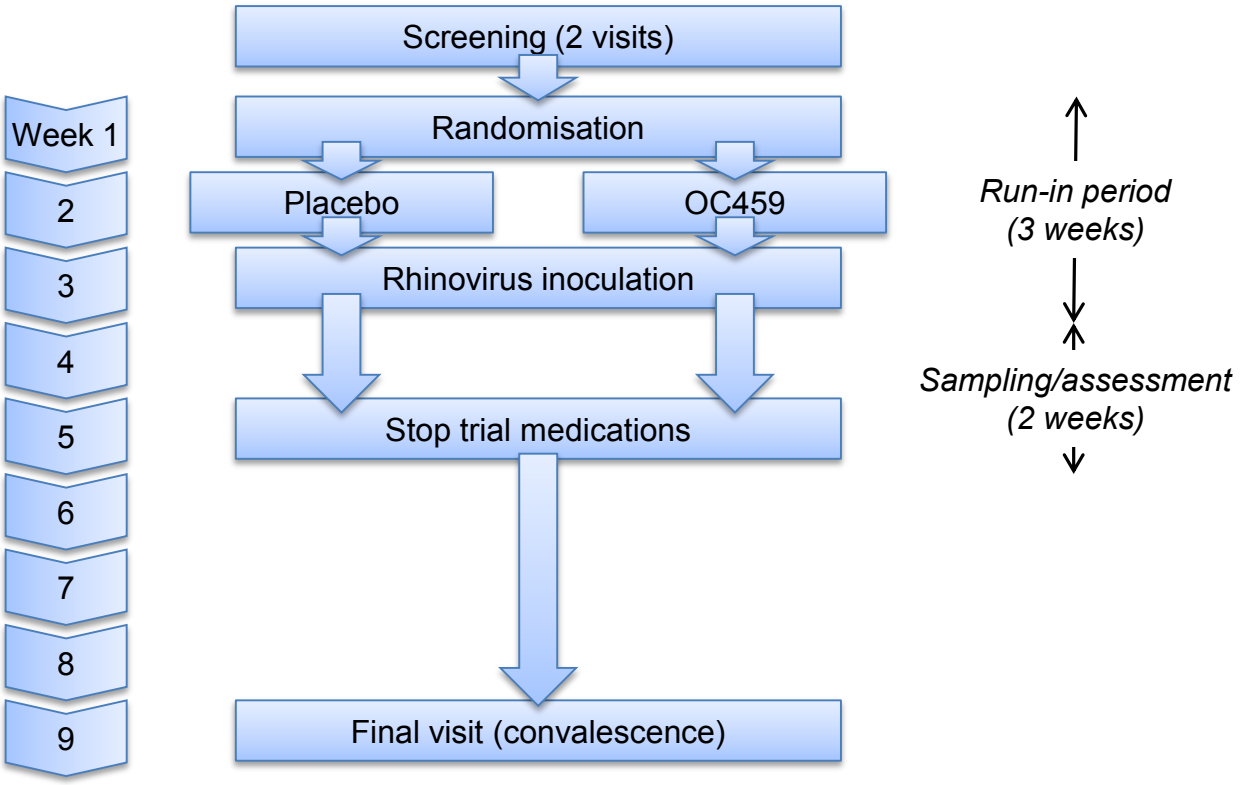
SOB	Shortness Of Breath
SUSAR	Suspected Unexpected Serious Adverse Reaction
$TCID_{50}$	Tissue culture infective dose = the amount of pathogenic agent to produce a pathological change in 50% of cell cultures inoculated
TLC	Total Lung Volume

KEYWORDS
Rhinovirus, Asthma, Exacerbation, PGD2, CRTH2

STUDY SUMMARY

TITLE	Effect of OC459 on the Response to Rhinovirus Challenge in Asthma.
DESIGN	Randomised, double-blind, placebo-controlled, parallel group study.
AIMS	To evaluate if the oral CRTH2 antagonist OC459 is effective in preventing and/or treating rhinovirus-induced asthma exacerbations.
OUTCOME MEASURES	Primary outcome: difference in symptom scores after rhinovirus infection. Secondary outcomes: measures of health status, lung function, airway hyper-responsiveness, exhaled nitric oxide (FeNO) levels, virus load, and other laboratory parameters (e.g. differential cell counts, levels of cytokine/biomarker proteins and mRNA in respiratory samples).
POPULATION	44 adults with asthma (of which 30 will be evaluable).
ELIGIBILITY	Adult subjects with moderate persistent asthma who meet the inclusion/exclusion criteria described.
TREATMENT	OC459 or placebo, for a total of 5 weeks per subject.
DURATION	27 months

FIGURE: STUDY OVERVIEW



1 INTRODUCTION

1.1 BACKGROUND

Importance of asthma and asthma exacerbations

Asthma is a long-term condition affecting the airways in the lungs, causing symptoms in patients such as breathlessness, wheeze, tightness in the chest and cough. Asthma is the most prevalent chronic respiratory disease, affecting 5-10% of adults with a further 30% of school age children reporting wheeze [1]. Although some children will outgrow their asthma, future prevalence is predicted to rise: the WHO estimate 300 million people worldwide are affected by asthma and that this is likely to increase to 400 million cases over the next 15-20 years [2].

The major morbidity, mortality and health care costs related to asthma are a result of periods of acutely increased symptomatology, called 'exacerbations' [3]. Acute exacerbations of asthma are an enormous problem to adults and children: ~5-10% of asthmatics have been hospitalised with an exacerbation and 60% have had an exacerbation in the previous year [4]. Asthma mortality in the UK is ~2,000 per annum – extrapolated to Europe, this means approximately ~25,000 people die unnecessarily of asthma each year. The financial cost of exacerbations is estimated to be ~50% of total expenditure on asthma care [5].

Lack of effective treatments for asthma exacerbations

Currently available therapies, predominantly inhaled corticosteroids and bronchodilators, prevent only ~40% of asthma exacerbations [6]. These figures are in the context of clinical trials – in real life asthma exacerbations are even less likely to be prevented: >50% of European asthma patients reported having an exacerbation in the last year with >1/3 children and >1/4 adults requiring urgent medical care visits as a result [7]. The vast majority of asthma exacerbations therefore continue to occur despite use of the best available current therapies.

When exacerbations do occur, treatment options are limited and have developed little in recent years. Treatment involves increasing doses of inhaled bronchodilators and systemic/oral steroids – more of the same drugs that failed to prevent the exacerbations occurring. Furthermore these treatments do not address the causes or mechanisms driving the exacerbations. Current preventive and therapeutic strategies are thus of limited efficacy and development of new approaches is urgently needed.

Causes and mechanisms of asthma exacerbations

The causes of asthma exacerbations are complex and likely to involve one or more of a number of factors including poor control of underlying disease, allergen exposure and viral infection. Respiratory viral infections appear the dominant individual precipitant as they are detected in the great majority of asthma exacerbations in both children (80-85%) and adults (60-80%) [8]. The most commonly detected viruses are human rhinoviruses (~60-80%), and it has been shown that experimentally infected asthmatics with rhinovirus (RV) precipitates an acute exacerbation [9].

The use of experimental RV infection has enabled the study of the mechanisms of asthma exacerbations. These have shown that RV infection leads to increased levels of inflammatory mediators in asthmatic subjects relative to healthy controls (specifically 'type 2 cytokines' e.g. IL-4, IL-5 and IL-13, as well as IL-33 and Prostaglandin D_2), and that these increases correlate with the severity of respiratory symptoms [10]. New treatments have subsequently been developed to targeting those mediators, e.g. an antibody against IL-5 [11].

Studies *in vitro* have found that the binding of Prostaglandin D_2 (PGD_2) to the CRTH2 receptor on inflammatory cells (e.g. Th2 lymphocytes, type 2 innate lymphoid cells) stimulates them to produce the type 2 cytokines implicated in asthma exacerbations [12, 13]. Thus treatment with a CRTH2 antagonist represents an attractive approach to prevent RV-mediated exacerbations in asthmatic patients.

1.2 RATIONALE FOR CURRENT STUDY

Hypothesis: This trial is designed to investigate the hypothesis that CRTH2 antagonism can prevent or attenuate the deterioration in symptoms in patients with asthma (i.e. asthma exacerbations) following rhinovirus infection.

Rationale for using the RV-induced asthma exacerbation model

The RV-induced asthma exacerbation model offers the ability to investigate treatment effects on exacerbations with a small number of volunteers, minimising the numbers exposed to a novel agent with limited safety data. In contrast, trials of therapies powered to evaluate an effect on naturally occurring exacerbations require several hundred volunteers, a long study period to capture enough events and are significantly more expensive to carry out.

The RV-induced exacerbation model has been used extensively around the world over >15 years, including four studies by Professor Johnston's group [9, 10, 14, 15], and is well-established with an excellent safety profile (reviewed in [16]); across the studies at Imperial there have been no adverse events, and no need for hospitalisation or systemic corticosteroids during the infection. Moreover, the RV-induced exacerbation model uniquely provides the opportunity to select a well-defined sample population, use a uniform viral challenge, and sample repeatedly as the exacerbation evolves, allowing accurate dissection of the timings of responses and their relationship to viral load.

Rationale for using the CRTH2 antagonist OC459

As of August 2013, at least 12 CRTH2 antagonists were in active clinical development [17]. Of these, OC459 (Atopix Therapeutics Ltd) had been trialled for the longest duration in a phase 2 study (17 weeks). In total, 637 patients across five published studies have been exposed to OC459 [18-22], including 482 patients with asthma, with an excellent safety record. OC459 has therefore been chosen for further study from the available CRTH2 antagonists due to its safety profile; there is no reason to think that is more or less effective than any other CRTH2 antagonist.

In addition, the largest study of OC459 to date suggested that it may be effective in reducing exacerbations: a trend towards reduction in asthma exacerbations was noted, with a 3.8% incidence in the pooled dose groups compared to 7.7% on placebo (p=0.107) [22]. A dose of 25 mg once daily was as effective as the other doses (100mg twice daily and 200mg once daily) in this study.

2 STUDY OBJECTIVES

Primary objective:

- To assess the impact of OC459 on Total Lower Respiratory Tract Symptoms Score after rhinovirus infection (sum of daily scores day 0 to day 14)

Secondary objectives:

- To assess the impact of OC459 on other clinical and laboratory parameters during rhinovirus infection:
  - Health status (assessed by Asthma Control Questionnaire (ACQ) scores)
  - Lung function (assessed by spirometry)
  - Airway inflammation (assessed by exhaled nitric oxide (FeNO) levels)
  - Airway hyperresponsiveness (assessed as the provocation concentration of histamine producing a 20% fall in FEV_1, or PC_{20})
  - Viral load (measured by quantitative Polymerase Chain Reaction (qPCR) on nasal lavage samples)
- To determine the mechanism of action of OC459, by assessing the impact of OC459 on the following during rhinovirus infection:
  - Eosinophil recruitment (measured by cell counts in sputum and bronchoalveolar lavage (BAL); eosinophil products, e.g. Eosinophil Cationic Protein (ECP), in BAL)
  - Recruitment of other immune cells (e.g. ILC2, Th2) implicated in the prostaglandin D_2 / type 2 inflammatory pathway to the airways and/or airway mucosa (measured by flow cytometry to sort and count cells in BAL, serum and nasal scrapes; and immunohistochemistry staining of bronchial biopsies)
  - Release of inflammatory mediators implicated in the prostaglandin D_2 / type 2 inflammatory pathway (e.g. the type 2 cytokines IL-4, IL-5, IL-13; measured by multiplex immunoassay on nasosorption, nasal lavage, bronchosorption and BAL, and mRNA analysis of cells in BAL and biopsies)
- To assess whether OC459 is able to restore deficient innate antiviral responses
  - Production of cytokines (e.g. interferon (IFN) α and λ) and expression of cytokine mRNA in cells from samples (blood, BAL) and infected with rhinovirus *ex vivo*
- To assess the safety of OC459
  - Adverse event reporting, physical examinations, vital signs, and clinical laboratory parameters

3 STUDY DESIGN

Type of study: This is a phase 2a, randomised, double-blind, placebo-controlled, parallel group study.

Study duration: The trial will take place over a 33 month period. On an individual basis, the trial will last for 9 weeks from randomisation. The trial will be considered complete when the last patient recruited completes the final visit.

Population size: 44 asthmatic subjects.

3.1 STUDY OUTCOME MEASURES

Primary outcome:

- Difference in Total Lower Respiratory Tract Symptoms Score after RV infection (sum of daily scores day 0 to day 14) between OC459 and placebo groups.

Secondary outcomes:

- Difference between OC459 and placebo groups in the following (all measured as peak and change from baseline):
    - Increase in ACQ score (health status)
    - Reduction in Forced Expiratory Volume in 1 second (FEV_1) or Peak Expiratory Flow (PEF) on spirometry
    - Increase in FeNO
    - Decrease in PC_{20}
    - Virus load in nasal lavage – total (area under the curve) for days 2-5+7+10, as well as peak and difference from baseline
    - Increase in sputum eosinophilia
    - Leukocyte numbers (e.g. ILC2, Th2) in FACS-sorted BAL, serum and nasal scrapes, and in immunohistochemistry-stained biopsies
    - Cytokine levels (e.g. IL-4, IL-5, IL-13) in nasosorption, nasal lavage, bronchosorption, BAL
    - mRNA expression of same cytokines in cells in BAL and bronchial biopsies
    - In *ex vivo* studies, protein and mRNA levels of antiviral cytokines (e.g. IFN-α, IFN-λ) produced by cells from blood, BAL, and bronchial brushings in response to rhinovirus infection
- Difference in number of adverse events in OC459 and placebo groups

4 PARTICIPANT ENTRY

4.1 PRE-RANDOMISATION EVALUATIONS

Potential subjects for the study will be recruited from hospital outpatient clinics, GP surgeries, spirometry sessions/clinics, other research projects, and advertisements. They will attend the respiratory clinic at St Mary's Hospital, where the outline of the study will be explained to them and a Participant Information Sheet (PIS) provided. They will be given the opportunity to ask questions and be informed that their participation is voluntary. Subjects may decide they wish to have more time to consider taking part in the study, in which case a follow up appointment will be made, or they may continue with the screening process. When a subject has had enough time to consider their participation in this study, and only when they have agreed to take part, they will be asked to read, sign and date a consent form in the presence of the investigator who will also sign the consent form. A copy will be kept in the research file, a copy given to the patient and a copy put into their medical notes.

After signing a consent form, eligible subjects will undergo screening. Screening involves taking a medical history and examination, lung function testing, a histamine challenge, FeNO levels, skin prick testing, blood tests, a urine pregnancy test, and a chest radiograph to assess suitability against the defined inclusion and exclusion criteria. Blood tests include general screening for underlying illness, specifically full blood count, urea and electrolytes, liver function tests, coagulation, and C-reactive protein (CRP), as well as serology for rhinovirus 16 (RV-16). They will be matched to the inclusion/exclusion criteria, which have been maintained from previous studies using the rhinovirus-induced asthma exacerbation model.

4.2 INCLUSION CRITERIA

- Age 18-55 years
- Male or female
- Clinical diagnosis of asthma for at least 6 months prior to screening
- An Asthma Control Questionnaire (ACQ) Score >0.75
- Positive histamine challenge test (PC_{20} <8 µg/ml, or <12 µg/ml and bronchodilator response ≥ 12%)
- Worsening asthma symptoms with infection since last change in asthma therapy
- Positive skin prick test to common aeroallergens (e.g. animal epithelia, dust mite)
- Treatment comprising inhaled corticosteroids (ICS) or combination inhaler (Long-Acting Beta Agonist with ICS), with a daily ICS dose of at least 100mcg fluticasone or equivalent.
- Participant is willing for their GP to be informed of their participation.
- English speaker

4.3 EXCLUSION CRITERIA

- Presence of clinically significant diseases other than asthma (cardiovascular, renal, hepatic, gastrointestinal, haematological, pulmonary, neurological, genitourinary, autoimmune, endocrine, metabolic, neoplasia etc.), which, in the opinion of the investigator, may either put the patient at risk because of participation in the trial, or diseases which may influence the results of the study or the patient's ability to take part in it
- Smoking history over past 12 months.
- Seasonal allergic rhinitis symptoms at screening or during the 3 week run-in (prior to rhinovirus inoculation).

- Asthma exacerbation or viral illness within the previous 6 weeks or during the 3 week run-in (prior to rhinovirus inoculation).
- Current or concomitant use of oral steroids, anti-leukotrienes or monoclonal antibodies.
- Pregnant or breast-feeding women. Patients should not be enrolled if they plan to become pregnant during the time of study participation (see note regarding contraception below).
- Contact with infants <6 months or immunocompromised persons, elderly and infirm at home or at work.
- Subjects who have known evidence of lack of adherence to medications and/or ability to follow physician's recommendations.

Contraception: To prevent pregnancy, male subjects with female partners of child bearing potential and female subjects must use at least two forms of adequate contraception for the entire duration of study participation. Adequate contraceptive precautions include:

1. Established use of oral, injected or implanted hormonal methods of contraception.
2. Placement of an intrauterine device (IUD) or intrauterine system (IUS).
3. Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/suppository.
4. Male sterilisation (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate).

Men whose partners are already pregnant must continue to use contraception i.e. a condom during the trial and for three months thereafter. The chosen contraception method(s) must be followed from the first Investigational Medicinal Product (IMP) administration until at least 3 months after receiving the last dose of the IMP.

Sperm donation: Male subjects must not donate sperm throughout the study and for at least 3 months after the final dose of IMP.

4.4 WITHDRAWAL CRITERIA

Participants will be free to discontinue trial medications at any point during the study without giving reason. Where possible, follow up will continue. If a participant chooses to withdraw consent, data up to the point of self-withdrawal will be included unless the participant chooses otherwise.

Investigators will withdraw any subject if they are unable to tolerate the study medications, or if the a research investigator feels this is necessary or appropriate. In all cases, the reason for withdrawal must be recorded on the case report form and in the subject's medical records. Subjects will be closely monitored by the Clinical Research Fellow. This is defined further in the section on 'Serious Adverse Events'.

Subjects who are considered evaluable (i.e. confirmed rhinovirus infection) with sufficient data to assess the primary endpoint (i.e. complete diary cards of symptoms up to day 14 post-rhinovirus infection) will be included in the analysis. Subjects who are either withdrawn from the trial or who chose to discontinue participation and do not meet these criteria will be replaced until a sufficient number of evaluable subjects are reached (n=20).

5 RANDOMISATION AND ENROLMENT PROCEDURE

5.1 RANDOMISATION PRACTICALITIES

Subjects who meet the relevant criteria and provide informed consent will be randomised to either OC459 or placebo in a 1:1 ratio. Randomisation will occur at a baseline visit after the screening visit(s), and will be in blocks of four in order to balance the number of patients allocated to each treatment group. An unblinded randomisation list (taking into account the blocking requirements) will be generated by Sealed Envelope Ltd and entered into the study database. This file will be password protected, with unblinding instructions provided by the database development team to the investigators.

At randomisation, the database will be interrogated and each new subject assigned the next sequential randomisation item on the list. The unblinded randomisation list will also be provided to the manufacturer, Atopix Ltd, in order to label IMP/placebo appropriately prior to dispensing to pharmacy. Thus the investigators, pharmacy and subjects will all be blinded.

5.2 UNBLINDING

Ceasing treatment rather than unblinding will be encouraged as far as possible. However, where knowledge of the treatment will assist in management of acute illness / emergency treatment, unblinding will be supported. The decision to unblind will be made by the Chief Investigator, with the Trial Steering Committee convened *ad hoc* if necessary to support the decision. Study participants will be provided with a Patient Alert Card indicating the study number and a 24 hour emergency contact number.

Unblinding will take place in line with the standard operating procedure used with the database. Specifically, once the decision has been made to unblind a subject, one of the study team will access the database and enter the subject details and study drug code. As soon as this is entered into the system, an email will be sent to the Trial e-mail address, the trial statistician, the Chief Investigator and the database team warning of a potential unblinding. The essential subject information will be checked against the data in the database and queries raised if they do not agree. The unblinding will not be actioned if there is a discrepancy between the data stored and the data entered. Once this information is accurately completed, a second form must be filled in to confirm the unblinding. The unblinded information will then be displayed, and a second email sent to the Trial e-mail address, the trial statistician, the Chief Investigator and the database team confirming the subject has been unblinded. This email will not continue details of the unblinded treatment. If the site unblinding account has been used, the database support team will set a new, pre-expired password and send new unblinding instructions to the study team.

The Chief Investigator will review the cause and results of the unblinding process with the study team, and possibly the Trial statistician, and document/implement any follow-up actions.

6 TREATMENTS

6.1 TREATMENT ARMS

The Investigational Medicinal Product (IMP) used in the study will be OC459 50mg or matched placebo film-coated tablets. The dose of OC459/placebo will be 50mg (one tablet) to be taken orally, once daily, for 5 weeks in total. Both OC459 and placebo will be manufactured and delivered by Atopix Therapeutics Ltd at no cost to the study. These will be supplied to the pharmacy at St Mary's Hospital to be stored securely.

The identity of the study medications will be blinded and packaged according to the randomisation schedule.

7 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

7.1 IMP DESCRIPTION

OC459 is a once-daily oral, highly potent and selective CRTH2 antagonist which is active on both the recombinant and native human receptor.

The integrated safety database for OC459 includes data for a total of 754 subjects who received OC459 for up to 3 months with more than 500 subjects receiving doses ≥100mg daily. The overall incidence of adverse events was similar for those treated with OC459 (28.2%) compared to placebo (26.7%) and few subjects in either group had serious adverse events (0.1% and 0.5% for OC459 and placebo respectively). The adverse event profile after OC459 administration was generally comparable to that after placebo; the most common adverse event reported was headache (6.6% vs 5.1% for OC459 and placebo, respectively).

OC459 repeat doses of up to 400mg have been studied in patients and no clear dose ordered effects on safety have been observed. Furthermore, no end organ toxicology or significant changes in clinical chemistry (including liver enzymes) have been observed in non-clinical toxicology studies including long term studies conducted at very high doses in the mouse (3 month dosing), rat (six months dosing) and the dog (12 month dosing). The known metabolites of OC459 have been detected in at least one species including the acylglucuronide of OC459 which is the major metabolite in humans.

7.2 STORAGE OF IMP

Clinical trial supplies will be stored below 30ºC, protected from moisture and light, in the pharmacy or other secure area within the investigational site. They must not be frozen or refrigerated. They will be stored in such a way that they cannot be mixed up or confused with other medications, be they clinical trial supplies or medicines for routine clinical use. In addition, returned study medication will not be stored with unused study medication.

7.3 DISPENSING AND ACCOUNTABILITY

A record of study drug movements will be kept to maintain accountability. Only authorised personnel (as designated by the study delegation log) will be allowed to distribute the drugs. Any queries regarding supply should be directed to the study coordinator or the lead trial pharmacist (contact details in the Appendices).

7.4 COMPLIANCE WITH TRIAL TREATMENT

Compliance will be self-reported by participants in their diary cards. In addition, we will collect and count returned tablets at the end of the study. Non-compliance will be defined as >20% missed doses as per diary cards and/or tablet count at the end of the study. Participants who are non-compliant will be excluded from a per protocol analysis.

7.5 POST-TRIAL TREATMENT

The IMP is unlicensed and therefore will not be made available to participants beyond the end of the study.

8 PHARMACOVIGILANCE

8.1 DEFINITIONS

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

Adverse Reaction (AR): all untoward and unintended responses to an IMP related to any dose administered. *All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.*

Unexpected Adverse Reaction: an AR, the nature or severity of which is not consistent with the applicable product information (eg investigator's brochure for an unapproved investigational product or summary of product characteristics (SmPC) for an authorised product*). When the outcome of the adverse reaction is not consistent with the applicable product information this adverse reaction should be considered as unexpected. Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.*

Serious Adverse Event (SAE) or **Serious Adverse Reaction:** any untoward medical occurrence or effect that at any dose:

- **Results in death.**
- **Is life-threatening** – *refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.*
- **Requires hospitalisation, or prolongation of existing inpatients' hospitalisation.**
- **Results in persistent or significant disability or incapacity.**
- **Is a congenital anomaly or birth defect.**

Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): any suspected adverse reaction related to an IMP that is both unexpected and serious.

8.2 CAUSALITY

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the study coordination centre who will notify the Chief Investigators. The pharmaceutical companies and/or other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

Relationship	Description
Unrelated	There is no evidence of any causal relationship
Unlikely	There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).
Possible	There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).
Probable	There is evidence to suggest a causal relationship and the influence of other factors is unlikely.
Definitely	There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.
Not assessable	There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.

8.3 REPORTING PROCEDURES

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the study coordination centre in the first instance. A flowchart is given below to aid in the reporting procedures.

8.3.1 Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form and sent to the study coordination centre within one month of the form being due.

8.3.2 Serious AR/AEs

Fatal or life threatening SAEs and SUSARs should be reported on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). The responsible investigator should sign the causality of the event. Additional information should be sent within 5 days if the reaction has not resolved at the time of reporting.

SAEs

An SAE form should be completed and faxed to the study coordination centre for all SAEs within 24 hours.

SUSARs

In the case of suspected unexpected serious adverse reactions, the staff at the site should:

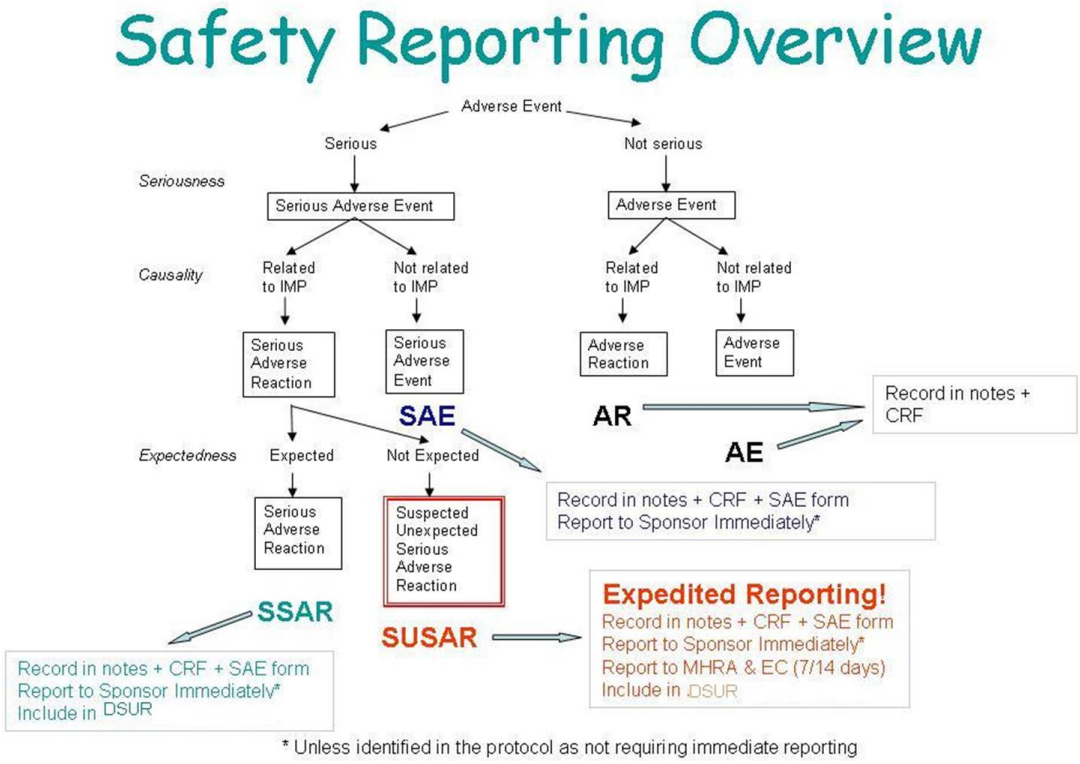
Complete the SAE case report form & send it immediately (within 24 hours, preferably by fax), signed and dated to the study coordination centre together with relevant treatment forms and anonymised copies of all relevant investigations.

Or

Contact the study coordination centre by phone and then send the completed SAE form to the study coordination centre within the following 24 hours as above.

The study coordination centre will notify the MHRA, REC and the Sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Local investigators should report any SUSARs and /or SAEs as required by their Local Research Ethics Committee and/or Research & Development Office.



Contact details for reporting SAEs and SUSARs
Fax: 020 7262 8913, attention Dr Hugo Farne
Email: asthma.trial@imperial.ac.uk
Tel: 020 7594 3764 (Mon to Fri 09.00 – 17.00)

9 ASSESSMENT AND FOLLOW-UP

9.1 SUMMARY OF STUDY ASSESSMENTS AND PROCEDURES

After screening and providing informed consent, eligible participants will undergo baseline investigations and randomisation, followed by a 21 day run-in period on placebo or OC459. Towards the end of this period they will have a baseline bronchoscopy and other investigations. At the end of the run-in period, subjects will have a further set of investigations prior to rhinovirus inoculation the same day. Regular sampling and assessments will take place as indicated whilst treatment administration continues uninterrupted for a further 14 days, for a total of 5 consecutive treatment weeks. Trial medications are then stopped (day 14). Subjects will attend for a final follow-up visit 4 weeks later with convalescence investigations (day 42). Table 1 summarises subject visits and procedures during the study. Details of the individual study procedures are listed below.

Study participants will be seen frequently during the study period, daily if they wish. They have details to contact the Clinical Research Fellow and Clinical Research Nurse, and will be offered daily telephone contact. In this way participants will be assessed regularly by the investigating team and any adverse events detected rapidly. When the study is completed, subjects will return to the care of their GP (they will not be routinely followed-up by the study group).

	Screening visit 1	Screening visit 2	Visit 1 (baseline)	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10	Visit 11
Study day			-21	-9	-8	0	2	3	4	5	7	10	42
Ideal weekday			F	W	T	F	Su	M	T	W	F	M	F
Visit length	30 min	60 min	60 min	45 min	3 hr	60 min	45 min	45 min	45 min	3 hr	60 min	45 min	30 min
Skin prick test	X												
Viral serology	X												X
Asthma Control Questionnaire (ACQ)	X		X	X		X						X	X
Spirometry (in clinic)	X		X	X		X	X	X	X	X	X	X	X
Histamine challenge (PC_{20})		X	X	X							X		
Exhaled nitric oxide (FeNO)		X	X	X		X		X		X	X	X	X
Blood tests		X	X		X			X		X		X	X
ECG		X											
Urine pregnancy test		X											
Chest radiograph		X											
IMP or placebo administration			Daily from day -21 →							day 14 (then stop)			
Nasosorption			X			X	X	X	X	X	X	X	
Nasal lavage			X			X	X	X	X	X	X	X	
Nasal scrape			X		X			X		X			
Bronchoscopy (bronchosorption, BAL, brushings, biopsies)					X					X			
Sputum induction			X	X					X		X		
Virus inoculation						X							
Symptom diaries including spirometry and medication			Daily at home during study period →										
Spirometry (portable, at home)			Daily at home during study period →										

Table 1: Summary of study visits and key procedures

9.2 SKIN PRICK TESTING

Atopic status will be determined by skin prick testing to a range of 10 common aeroallergens. Positive histamine / negative diluent controls will be included.

- Mixed grass pollen
- House dust mite (Der P)

- Silver birch
- 3 trees
- Cat dander
- Dog hair
- Mugwort
- Aspergillus fumigatus
- Cladasporium herbarum
- Alternaria alternata

≥ 1 positive reaction (wheal 3 mm greater than the negative control) will be considered diagnostic of atopy.

9.3 BLOOD (INCLUDING VIRAL SEROLOGY)

Screening visit blood (in tubes with ethylenediamine tetraacetic acid (EDTA), citrate, or heparin) will be taken for full blood count, renal function, liver function tests, glucose, clotting, CRP, vitamin D and total IgE. These will be processed in the Haematology and Chemical Pathology Laboratories of St Mary's Hospital.

Blood will also be taken on study visits as per Table 1; the specific samples taken on each visit are outlined in Table 2. Serology will be performed at screening and day 42 for detection of antibodies to rhinovirus-16 (RV-16). Serum will also be used to count cell types in the circulating blood using flow cytometry, and to assess the immune response to rhinovirus *ex vivo*.

Study day	Full blood count (EDTA 4.0mL)	Clotting (Citrate 2.7mL)	Biochem + IgE (Heparin 3.5mL)	Serum
Screening	x1	x1	x2	6mL
Day -21				80mL
Day -7	x1	x1	x2	80mL
Day 3	x1	x1	x2	10mL
Day 5	x1	x1	x2	80mL
Day 10	x1	x1	x2	10mL
Day 42	x1	x1	x2	6mL
Total (mL)	**24.0mL**	**16.2mL**	**42.0mL**	**292mL**

Table 2: Overview of blood, plasma, and serum requirements (mL)

9.4 CLINICAL SYMPTOM SCORES (INCLUDING ACQ)

Patients will be required to keep a diary card of both upper and lower respiratory tract symptoms during the study period (see Appendices). The diary cards will be commenced 21 days prior to inoculation (on day -21) to derive a baseline symptom score. Symptom scores will be kept daily for 6 weeks post-inoculation until the convalescent visit. Spirometry measurements and drug compliance will be recorded on the same diary cards.

<u>Upper Respiratory Tract Symptoms</u>
The diary card of upper respiratory tract symptoms will be started 21 days prior to inoculation. A total upper respiratory clinical symptom score will be derived using a scale of 0 to 3 (absent, mild, moderate and severe) for each of the following eight respiratory symptoms: sneezing, headache, malaise, fever/chills, nasal discharge, nasal obstruction, sore throat and cough, according to previously established criteria [23]. Symptoms will be recorded at the same time of day and before any procedures such as bronchoscopy are performed. An example is shown below:

Symptom	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Sneezing							
Headache							
Malaise							

Fever/chills							
Nasal discharge							
Nasal obstruction							
Sore throat							
Cough							
Total score							

0 = absent, 1 = mild, 2 = moderate, 3 = severe

The maximum clinical severity score on any one day would be 24. Individual symptom scores will be accumulated over the six-day period from the onset of the cold and the recording on the day prior to inoculation subtracted. Thus, for a patient who has a score of zero on day -1 prior to inoculation, the maximum cumulative score for the six days is 144.

Lower Respiratory Tract Symptoms

A diary card of lower respiratory tract symptoms will be completed with a scoring system as outlined below:

Symptom	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Cough on waking							
Wheeze on waking							
Daytime cough							
Daytime wheeze							
Daytime breathlessness							
Nocturnal cough, wheeze, breathlessness							
Total score							

0 = absent, 1 = mild, 2 = moderate, 3 = severe

An exacerbation of asthma is defined as an increase in the total respiratory score of at least 2 points over baseline on 2 consecutive days.

Asthma Control Questionnaire (ACQ)

The ACQ is a standard measure of disease severity and combines symptom scores with objective parameters such as FEV_1 predicted and medication use. The ACQ should be interviewer administered. Further details are in the appendices. ACQ scoring will be assessed by study team on study visits as per Table 1.

9.5 SPIROMETRY

Spirometry will be performed using a MicroLab spirometer (CareFusion, Kent, UK) on study visits as per Table 1. At the screening visit measurements of Forced Expiratory Volume in 1 second (FEV_1) and Forced Vital Capacity (FVC) will be made:

- Posture must be consistent during a study, either standing or sitting, with no breathing limitation.
- The subject should breathe in to total lung capacity. A good tight seal by the lips round the mouthpiece is essential. The subject should then exhale forcibly into the spirometer, blowing as hard as possible and continue to residual volume.
- The best value of 3 attempts will be recorded.

The predicted or reference values for lung function measurements are those recommended by the Report Working Party for the European Community for Coal and Steel. Also incorporated are the recommendations of the British Thoracic Society (BTS) and the Association of Respiratory Technicians and Physiologists.

Reversibility: At screening, spirometry will be repeated 15 minutes after the administration of 200µg salbutamol via metered dose inhaler (MDI) and volumatic spacer to assess reversibility. A cut-off point for significant reversibility of 12% of FEV_1 predicted and 200ml will be taken as supportive of a diagnosis of asthma. Clinic spirometry will be carried out as part of the protocol for induced sputum.

Home spirometry: The subjects will be provided with a portable PiKo-1 spirometer (nSpire Health, Hertford, UK) to carry out daily measurements of FEV_1, FVC and PEF at home. At the baseline visit the subjects will be instructed in the use of the spirometer and will commence measurements to gain familiarity with its use. Measurements will be continued daily for the 6 weeks after inoculation.

9.6 HISTAMINE CHALLENGE (PC_{20})

Histamine challenge (maximum concentration 16 mg/mL) will be performed according to American Thoracic Society (ATS) guidelines by using the 2 minute tidal breathing method [24]. The provocative concentration of histamine causing a 20% reduction in FEV_1 (PC_{20}) is then calculated.

9.7 EXHALED NITRIC OXIDE (FENO)

Measurement of FeNO levels will be performed using a NIOX VERO device (Aerocrine, Solna, Sweden) with single use patient filters on study visits as per Table 1, and prior to all other assessments. The patient should not eat or drink or participate in any strenuous physical activity for at least one hour prior to testing. The measurement of FeNO will be performed according to American Thoracic Society (ATS) guidelines [25]:

- Patients should be seated comfortably
- Explain test procedure to patient, emphasisng that it is a relaxed manoeuvre
- Instruct patient to
    1. Empty their lungs by breathing out thoroughly
    2. Close their lips around the mouthpiece on the patient filter so that no air leakage occurs
    3. Inhale deeply through the patient filter to Total Lung Capacity (TLC)
    4. Exhale slowly through the filter using the display as a guide to the required exhalation pressure and duration

The instrument will analyse the sample and generate a result in parts per billion (PPB) in approximately one minute.

9.8 ELECTROCARDIOGRAM (ECG)

All patients eligible to participate in the study will have a single electrocardiogram (ECG), as part of routine assessment for any medical abnormalities.

9.9 URINE PREGNANCY TEST

All female patients eligible to participate in the study will have a single urine pregnancy test.

9.10 CHEST RADIOGRAPH

All patients eligible to participate in the study will have a single chest radiograph performed prior to their bronchoscopy, as per our local research bronchoscopy guidelines. The radiation dose associated with a single chest radiograph is minimal (0.02mSv, equivalent to 3 days natural

background radiation) and the risk of adverse effects is negligible (risk of inducing a cancer ~1:1,000,000).

9.11 NASOSORPTION

This minimally invasive technique samples nasal (i.e. upper airway) mucosal lining fluid and offers potential benefits over nasal lavage including improved sensitivity through avoidance of significant analyte dilution (inherent with lavage). Nasosorption will be performed on study visits as per Table 1, prior to bronchoscopy and nasal lavage to avoid contamination by the local anaesthetic (bronchoscopy) or saline (nasal lavage) introduced into the nose.

Two strips of Synthetic Absorptive Matrix (SAM) (Leukosorb, Pall Life Sciences, Hampshire, UK) measuring 7mm x 35mm will be placed inside the participant's nostrils for 2 minutes to obtain samples of nasal lining fluid. This is a painless, minimally invasive procedure that will not require any local anaesthetic.

Each piece of SAM will be placed in a single labelled Spin-X Centrifuge Tube with Filter (Sigma-Aldrich Cat no: CLS8161) containing an assay buffer. This will be transported to the lab on ice for initial processing and transferred to -80°C pending analysis for proteins and cytokines by multiplex immunoassay (e.g. using the Meso Scale Device, MSD).

9.12 NASAL LAVAGE

Nasal lavage is performed on study visits as per Table 1 using the following technique:

- With subjects head extended and soft palate closed, up to 10mL of sterile normal saline is instilled into each nostril using a Pasteur pipette.
- Subjects then gently blow their nose into a sterile universal container or sputum pot.
- Lavage fluid is stored on ice, vortexed and aliquoted for storage at -80°C.

Nasal lavage fluid will be analysed to quantify the degree of viral shedding and determine viral load. Nasal lavage samples pre- and post-rhinovirus inoculation will also be tested for all common respiratory viruses by a PCR panel to exclude coincidental infections. Where possible, nasal lavage fluid will also be analysed for proteins and cytokines by multiplex immunoassay.

9.13 NASAL SCRAPE

Up to four nasal scrapes will be performed on study visits as per Table 1 to obtain nasal epithelial cells. All nasal scrapes will be performed after the collection of nasosorption and nasal lavage samples. They will be collected using the following technique:

- A careful examination of the nose is made to identify the nasal mucosa on the inferior turbinate. A plastic nasal curette (Rhinoprobe, Arlington Scientific) is advanced into the nostril until the tip is placed on the surface of the inferior turbinate. Using a gentle scraping motion a tissue sample is collected. This method of sampling is almost painless, and has been extensively employed in adults and infants.
- Nasal scrapes will be placed in labelled sterile scrape tubes with culture media or flow buffer, for culture and flow cytometry respectively.

9.14 BRONCHOSCOPY

Bronchoscopies will be performed on study visits as per Table 1 in the endoscopy suite at St Mary's Hospital, in accordance with British Thoracic Society (BTS) guidelines [26]:

- Subjects will sign a consent form on entering the study and will sign a separate consent form for each bronchoscopy. The procedure will be explained during the assessment stage and subjects will be given written information specifically regarding bronchoscopies in addition to the Participant Information Sheet.
- Subjects will fast for six hours prior to the procedure.
- Resuscitation equipment (for intubation, ECG monitoring and defibrillation) and necessary drugs (salbutamol, theophylline, adrenaline, hydrocortisone) will be available in the bronchoscopy room.
- Premedication will be given including:
  - Nebulised salbutamol 2.5mg prior to bronchoscopy
  - Sedation with midazolam (2-10mg) and/or fentanyl (25-200mcg) as necessary
  - Lignocaine spray (10%) and solutions (1%) for topical anaesthesia. The total dose will not exceed 7mg/kg or 400mg in total.
- Supplemental oxygen at a rate of $2Lmin^{-1}$ is given via a nasal cannula and oxygen saturations and heart rate are monitored with a pulse oximeter continuously. Intravenous access will be mandatory in all cases.
- The subject will be monitored during the bronchoscopy by a separate nurse and a second suitably qualified physician will be present to act as an advocate.
- The following samples will be collected in this order:
  1. Bronchosorption using Synthetic Absorptive Matrix (SAM)
  2. Bronchial brushings
  3. Bronchoalveolar lavage (BAL)
  4. Bronchial biopsy

  At the second bronchoscopy, sampling should be performed on the contralateral side to the analogous procedures done at the first bronchoscopy.
- All adverse events (pain, bleeding, hypoxia etc) will be recorded.
- Subjects will be observed for 1-2 hours, nil by mouth, after the procedure.
- Transportation will be arranged as subjects should not drive on the day of the procedure.
- All subjects will have a contact telephone number on discharge.

9.14.1 Bronchoscorption using Synthetic Absorptive Matrix (SAM)

Bronchosorption (Mucosal Diagnostics, Hunt Developments Ltd, Midhurst, UK) is a technique to sample bronchial mucosal lining fluid. The main benefit of this technique is the measurement of previously undetectable mediators through avoidance of the significant analyte dilution associated with bronchoalveolar lavage.

- The bronchosorption device is passed down the operating port of the bronchoscope. The distal end of the inner probe incorporates a folded strip of Leukosorb (Pall Life Sciences, Hampshire, UK) measuring 1.8mm x 30mm which are placed on either the right, or left (chosen at random) lower lobe bronchial mucosa for 30 seconds.
- Following sampling, the bronchosorption device is withdrawn back into its sheath and the complete device is removed from the bronchoscope.
- The sampling end of the probe is cut off and treated in an identical way to the nasosorption strips of Leukosorb.

Proteins and cytokines will be measured in the samples (e.g. IL-4, IL-5 and IL-13 by multiplex immunoassay such as MesoScale Discovery (MSD) Th1/Th2 7-plex).

9.14.2 Bronchial brushings

Up to six bronchial brushings will be taken from the lower or middle lobe sub-segmental bronchi with a standard disposable cytology brush at each bronchoscopy:

- Deploy each brush at segmental or sub-segmental carina. Advance 1-2cm but keep the brush in sight. Gently brush the side-wall avoiding any bleeding. If a brush has blood on

it, dispose and flush the bronchoscopic channel with a small volume (2-5ml of sterile saline before proceeding with additional brushes.
- Place each brush in a 2ml screw cap tube containing 1mL RNAse-free PBS on ice. Cut the wire and close the cap over the brush.
- Store brushing tubes on ice until further processing.

Cells are removed from the brushes and split into samples to be either cultured for *ex vivo* studies, or used for RNA extraction and analysis.

9.14.3 Bronchoalveolar lavage (BAL)

- The bronchoscope is gently inserted into one segmental bronchus of either the right or left middle lobes.
- BAL is performed by instillation of sterile physiological (0.9%) saline at room temperature in 30ml aliquots to a total of 180-240ml, aiming for a return of ~100ml.
- The BAL fluid is collected into a plastic chamber and transferred to polypropylene tubes for transport on ice to the laboratory.

Samples will be centrifuged to separate the cells and supernatant. The cells will be subject to mRNA analysis and flow cytometry, to identify and separate the different inflammatory cell phenotypes. The supernatants will be analysed for proteins and cytokines (where they are present in sufficient concentrations).

9.14.4 Bronchial biopsy

Six bronchial biopsies will be taken from the segmental and sub-segmental bronchi of the right lower and middle lobe at each bronchoscopy, using Keymed 2mm biopsy channel cupped and fenestrated biopsy forceps [FB-19C-1 (1111065)]. Four biopsies will be placed in 4% paraformaldehyde for preparation of paraffin blocks. Immunohistochemistry staining will be used to detect different phenotypes of inflammatory cells. The remaining two biopsies will be used for mRNA analysis.

9.15 SPUTUM INDUCTION

Sputum induction will be performed on study visits as per Table 1, in accordance with ERS guidelines[27].

- Subjects will be pre-medicated with 200µg salbutamol via metered dose inhaler and large volume spacer and baseline FEV_1 measured after 10 minutes.
- If the subject's FEV_1 is ≥60% of predicted, the following procedure should be used:
  - Administer 3-4.5% hypertonic saline via nebulizer.
  - Perform 2-5 minutes of inhalation then stop inhalation and check FEV_1 reading.
  - Repeat this process 3 times (i.e. a maximum of 20 minutes).
- If the subjects FEV_1 <60% of predicted, the following should be used:
  - Use normal saline (0.9%) initially.
  - Perform 30 seconds of inhalation then stop inhalation and check FEV_1 reading.
  - Repeat this process for 1 min and then 2 mins of inhalation.
  - Repeat this process with 3% saline with inhalations of 30 secs, 1 min and 2 mins.
  - Repeat this process with 3-4.5% hypertonic saline for 30 secs, 1, 2, 4, and 8 mins.
- In either case if the subject's FEV_1 drops by 20% at any time, or if the subject experiences excessive coughing, bronchoconstriction, hyperventilation, dyspnoea, dizziness, nausea and vomiting, or an increased wheeze, the induction should be stopped immediately and rescue therapy given.

Sputum plugs are selected from saliva by macroscopic inspection of the sample. An aliquot is analysed, unprocessed, for virus load by quantitative PCR. The remaining sample is processed

and centrifuged to separate the cells, which are then stained and counted for differential cell counts.

9.16 VIRUS INOCULATION

Details of the production and testing of the rhinovirus serotype 16 (RV-16) inoculum have been previously published [28]. This inoculum was prepared under the supervision of the Director of the Medical Research Council Common Cold Unit and was fully safety tested for adventitious agents according to published safety guidelines. It has been stored in individual unopened aliquots in the applicants -80°C freezer since production. It has been used in previous studies at high dose [9, 28-30] and low dose [31] over a time span >15 years. There have been no serious adverse events in any of these studies.

Subjects will be inoculated on a single occasion with stored inoculum, described above, divided equally between the two nostrils. A total dose of 100 $TCID_{50}$ (tissue culture infective dose; the amount of a pathogenic agent that will produce pathological change in 50% of cell cultures inoculated) is used. This is done slowly with sufficient interval between each spray to ensure maximum contact time between the spray and the nasal and pharyngeal mucosa. Subjects will be asked not to swallow during the procedure to ensure maximal pharyngeal contact. Subjects will also be asked to sniff at each actuation to encourage delivery of particles of inoculum to the lower airway. Identical methods have been used in our previous studies in COPD and asthmatic subjects.

9.17 LOSS TO FOLLOW-UP

Participants who are lost to follow up will not be replaced if they were evaluable (i.e. confirmed rhinovirus infection) and there is sufficient data to assess the primary outcome (i.e. complete diary card of symptoms to day 14 post-rhinovirus infection). Subjects who are lost to follow up and do not meet these criteria will be replaced until a sufficient number of evaluable subjects are reached (n=20).

9.18 TRIAL CLOSURE

The trial will be considered complete when the last patient recruited completes the final visit.

10 STATISTICS AND DATA ANALYSIS

10.1 SAMPLE SIZE CALCULATION

The sample size determination is based on the following assumptions:

Type I error probability α = 5%

Effect size = (μ1-μ0)/σ, where
μi = mean PEP in group i=(i=1: OC459, i=0: placebo),
σ = standard deviation of PEP, and
PEP is primary end-point (=total daily lower respiratory symptom scores over d0 to d14).

Based on a previously completed trial with similar design conducted at the same study site [10], σ is estimated to be 21.15 and the effect size equal to 22.21, yielding n=15 evaluable subjects per treatment group at 80% power. This is grossed-up for 80% rhinovirus inoculation success and adjusted for expected drop-outs to yield 22 enrolled patients per treatment group.

10.2 DATA ANALYSIS

Subjects completing the study protocol will be defined as infected or un-infected (neutralising antibody to RV-16 at 6 weeks post inoculation at a titre <1:4 and no detection of RV-16 by qPCR in nasal lavage, sputum or BAL at any point after inoculation). Only subjects with confirmed rhinovirus infection will be included in statistical analysis.

Quantitative assessments of symptom scores, lung function, exhaled nitric oxide (FeNO), airway hyperresponsiveness (histamine challenge, PC_{20}), virus load, inflammatory cell numbers and inflammatory markers will be compared within subjects to determine differences between baseline and during infection. Within-subject differences will be analyzed using ANOVA and 2-tailed paired Student's t-tests or Wilcoxon signed rank test as appropriate.

Differences between the patients taking OC459 and placebo group will be analyzed using unpaired parametric or non-parametric analysis as appropriate at each phase of the study. Correlations between (but not limited to) the following will be examined using Spearman's rank correlations to investigate possible causal relationships:

- illness severity (symptoms, lung function, airway hyperresponsiveness)
- virus load
- inflammatory cell counts
- inflammatory markers
- gene expression

Data and all appropriate documentation will be stored for a minimum of 10 years after the completion of the study, including the follow-up period.

11 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

11.1 RISK ASSESSMENT AND MONITORING

Regular monitoring will be performed by the Joint Research Compliance Office (JRCO) Clinical Trial Monitor at Imperial College, who will monitor the study regularly based on a completed risk assessment by the sponsor. The study will be monitored according to Good Clinical Practice (GCP). Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements.

11.2 SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial”.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the NHS host organisation within seven calendar days.

11.3 REPORTING

The Chief Investigator shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the Research Ethics Committee, host organisation, and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

12 REGULATORY ISSUES

12.1 CTA

Clinical Trials Authorisation will be obtained from the UK Competent Authority, the Medicines & Healthcare products Regulatory Agency (MHRA), prior to starting recruitment.

12.2 ETHICS APPROVAL

The Chief Investigator will obtain approval from a Research Ethics Committee. The study must be submitted for Site Specific Assessment (SSA) at each participating NHS Trust. The Study Coordination Centre will require a copy of the Trust R&D approval letter before accepting participants into the study. The study will be conducted in accordance with the recommendations for physicians involved in research on human subjects adopted by the 18th World Medical Assembly, Helsinki 1964 and later revisions.

12.3 CONSENT

Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Signed participant consent should be obtained. The right of the participant to refuse to participate without giving reasons must be respected. After the participant has entered the trial the clinician remains free to give alternative treatment to that specified in the protocol at any stage if he/she feels it is in the participant's best interest, but the reasons for doing so should be recorded. In these cases the participants remain within the study for the purposes of follow-up and data analysis. All participants are free to withdraw at any time from the protocol treatment without giving reasons and without prejudicing further treatment.

12.4 CONFIDENTIALITY

Participants' identification data will be required for the registration process. The Study Coordination Centre will preserve the confidentiality of participants taking part in the study and is registered under the Data Protection Act.

12.5 INDEMNITY

Imperial College London holds negligent harm and non-negligent harm insurance policies which apply to this study.

12.6 SPONSOR

Imperial College London will act as the main Sponsor for this study. Delegated responsibilities will be assigned to the NHS trusts taking part in this study.

12.7 FUNDING

The Medical Research Council (MRC) are funding this study.

12.8 EXPENSES AND BENEFITS

Participants in the study will have their travel expenses refunded. They will also be given a donation of £1,500 to compensate for time and inconvenience of taking part in the study. If subjects drop out, they will receive a pro rata amount: £300 if after the first bronchoscopy; £500 if after rhinovirus inoculation; £1,000 if after the second bronchoscopy; and £1,500 on completion.

12.9 AUDITS AND INSPECTIONS

The study may be subject to inspection and audit by Imperial College London under their remit as Sponsor, the Study Coordination Centre and other regulatory bodies to ensure adherence to GCP.

13 TRIAL MANAGEMENT

The day-to-day management of the trial will be co-ordinated by Dr Hugo Farne (Clinical Research Fellow), with close support from Dr David Jackson, Dr Ross Walton, and Professor Sebastian Johnston.

A Trial Steering Committee will be appointed and will be responsible for overseeing the progress of the trial. This will incorporate the activities of a Data Monitoring Committee. The Trial Steering Committee will include members of the research team, a patient representative, and a consultant in Respiratory medicine independent of the study group. An independent statistician will be invited as required. The Trial Steering Committee will meet every six months (and additionally on an *ad hoc* basis as required) to review the data, oversee the safety of subjects in the trial, and ensure that no events have occurred that would slow the progression of the trial. The minutes from these meetings will be stored in the Trial Management Folder.

14 PUBLICATION POLICY

It is expected that after analysis the data from this study will be widely distributed in the medical and scientific community. Facilitated with presentations at local, national and international meetings, we hope to publish widely in the medical literature. In addition, we have an excellent media department at Imperial College and publicise research that has public interest when it is published.

15 REFERENCES

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APPENDICES

Appendix A. Pharmacy Contact Details

Severine Rey
Lead trial pharmacy clinical trials technician
Outpatient Pharmacy Department
St Mary's Hospital
Imperial College Healthcare NHS Trust
Praed Street
London W2 1PG
Telephone: 020 3312 7643
Fax: 020 3312 6708

Appendix B. Participant daily diary card record

NAME ..... Please score symptoms in the boxes as shown: 0 = no symptoms 1 = mild 2 = moderate 3 = severe

Date														
Sneezing														
Runny nose														
Blocked or stuffy nose														
Sore throat or hoarse voice														
Headache or face pain														
Generally unwell														
Chill, fever or shivery														
Cough														
Total cold score														
Cough on waking														
Wheeze on waking														
Daytime Cough														
Daytime Wheeze														
Daytime chest tightness														
Daytime breathlessness														
Nocturnal cough, wheeze, breathlessness														
Total Chest score														
Medications (name and dose)														
1.														
2.														
3.														
Spirometry: Peak Flow am														
FEV_1 am														
Peak Flow pm														
FEV_1 pm														

Appendix C. Asthma Control Questionnaire (ACQ)

1. On average, during the past week, how often were you woken by your asthma during the night?

0	Never
1	Hardly ever
2	A few minutes
3	Several times
4	Many times
5	A great many times
6	Unable to sleep because of asthma

2. On average, during the past week, how bad were your asthma symptoms when you woke up in the morning?

0	No symptoms
1	Very mild symptoms
2	Mild symptoms
3	Moderate symptoms
4	Quite severe symptoms
5	Severe symptoms
6	Very severe symptoms

3. In general, during the past week, how limited were you in your activities because of your asthma?

0	Not limited at all
1	Very slightly limited
2	Slightly limited
3	Moderately limited
4	Very limited
5	Extremely limited
6	Totally limited

4. In general, during the past week, how much shortness of breath did you experience because of your asthma?

0	None
1	A very little
2	A little
3	A moderate amount
4	Quite a lot
5	A great deal
6	A very great deal

5. In general, during the past week, how much of the time did you wheeze?

0	Not at all
1	Hardly any of the time
2	A little of the time
3	A moderate amount of the time
4	A lot of the time
5	Most of the time
6	All the time

6. On average, during the past week, how many puffs of short-acting bronchodilator (eg. Ventolin) have you used each day?

0	None
1	1-2 puffs most days
2	3-4 puffs most days
3	5-8 puffs most days
4	9-12 puffs most days
5	13-16 puffs most days
6	More than 16 puffs most days

7. FEV_1 pre-bronchodilator:

0	>95% predicted
1	95-90%
2	89-80%
3	79-70%
4	69-60%
5	59-50%
6	<50% predicted