Statistical Analysis Plan (SAP)

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

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2.1. 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)

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- To assess the impact of OC459 on other clinical and laboratory parameters during rhinovirus infection in asthma:
    - Health status
    - Lung function
    - Airway inflammation
    - Airway hyper-responsiveness
    - Viral load
- To determine the mechanism of action of OC459, by assessing the impact of OC459 on the following during rhinovirus infection:
    - Eosinophil recruitment
    - Recruitment of other immune cells (e.g. ILC2, Th2) implicated in the prostaglandin D2 / type 2 inflammatory pathway to the airways and/or airway mucosa
    - Release of inflammatory mediators implicated in the prostaglandin D2 / type 2 inflammatory pathway (e.g. the type 2 cytokines IL-4, IL-5, IL-13)
- To assess the safety of OC459
- Exploratory - To assess whether OC459 is able to restore deficient innate antiviral responses in asthma

3. Study Endpoints

3.1. Primary Endpoint Variable

- Total Lower Respiratory Tract Symptoms Score after rhinovirus infection (sum of daily scores day 0 to day 14)

3.2. Secondary Endpoint Variables

- To assess the impact of OC459 on other clinical and laboratory parameters during rhinovirus infection in asthma:
  - Upper Respiratory Tract Symptoms Scores from D0 to D14
  - Corrected Lower Respiratory Tract Symptoms Scores from D0 to D14
  - Asthma Control Questionnaire (ACQ-6) scores
  - Spirometry – FEV_1, FVC, PEFR
  - Exhaled nitric oxide (FeNO) levels
  - Provocation concentration [of histamine] producing a 20% fall in FEV_1 (PC_{20})
  - Viral load (measured by qPCR)
- To determine the mechanism of action of OC459, by assessing the impact of OC459 on the following during rhinovirus infection in asthma:
  - cell counts in sputum and bronchoalveolar lavage (BAL)
  - immune cells (e.g. ILC2, Th2) in BAL, blood and nasal scrapes, measured by flow cytometry
  - immune cells in bronchial biopsies, measured by immunohistochemistry staining
  - soluble mediators in the upper and lower airways, measured by multiplex immunoassay on nasosorption, nasal lavage, bronchosorption and BAL samples
- To assess the safety of OC459
  - Adverse event reporting
  - physical examinations
  - vital signs
  - clinical laboratory parameters
- Exploratory - To assess whether OC459 is able to restore deficient innate antiviral responses
  - Production of antiviral interferons (IFN), e.g. β and λ by cells from blood and bronchial brushings following infection with respiratory viruses *ex vivo*
  - expression of mRNA for interferon and a panel of interferon-stimulated genes in cells from the same *ex vivo* studies

4. Background/Introduction

4.1. Introduction

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. 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'. 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. Asthma mortality in the UK is ~1,000 per annum – extrapolated to Europe, this means approximately ~11,000 people die unnecessarily of asthma each year. The financial cost of exacerbations is estimated to be ~50% of total expenditure on asthma care.

As of August 2013, at least 12 CRTH2 antagonists were in active clinical development. 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, 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 it 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). A dose of 25 mg once daily was as effective as the other doses (100mg twice daily and 200mg once daily) in this study.

Based on the above; 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.

4.2. Study Design

This is a randomised, double-blind, placebo-controlled, parallel group phase 2a study which will take place over a 36 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.

4.3. Study Population

English speaking, male or female patients between the age of 18-55 who have had a Clinical diagnosis of asthma for at least 6 months prior to screening will be deemed eligible for the trial if they satisfy the following additional Inclusion Criteria:

- An Asthma Control Questionnaire (ACQ-6) 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.

Patients must also not fall under any of the following 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.
- 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.

Whilst not listed within the trial protocol; an additional test was carried out to assess previous exposure to rhinovirus 16, as evidenced by the presence of serum neutralising antibodies to rhinovirus 16 at screening.

Subjects who meet the relevant criteria and provide informed consent are deemed eligible for randomisation to the trial.

4.4. Sample Size

An overall population size of 44 asthmatic subjects was based on the following assumptions:

- Type I error probability $\alpha = 5\%$
- Effect size = $(\mu 1-\mu 0)/\sigma$
    - μi = mean PEP in group i=(i=1: OC459, i=0: placebo),
    - σ = standard deviation of PEP
- PEP is primary end-point (=total daily lower respiratory symptom scores over D0 to D14, potential maximum of 315).

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.

4.5. Treatment Groups

- OC459: 50mg to be taken orally as one tablet, once daily, for 5 weeks in total.
- Placebo: to be taken orally as one tablet, once daily, for 5 weeks in total.

4.6. Randomisation

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. A dummy unblinded randomisation list (taking into account the blocking requirements) will be generated by the trial statistician and tested. Once tested, a statistician working independent to the trial will create the final list for entering 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.

4.7. Withdrawal and Replacement

Subjects who are considered evaluable (confirmed rhinovirus infection) and have completed at least 14 days post inoculation with RV-16 will be included in the analysis. Subjects lost to follow up will not be replaced if they were evaluable with sufficient data to assess the primary outcome. 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=30).

4.8. 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.

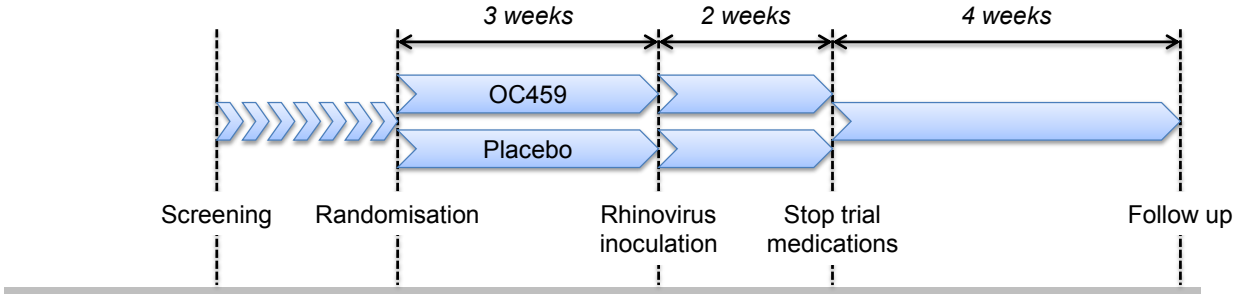
The Chief Investigator will review the cause and results of the unblinding process with the study team, and possibly the Trial statistician, and document/implement any follow-up actions.

4.9. Schedule of Time and Events

Table 1: Schedule of Visits and Events

	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 2: Schedule of Treatment and Events



Day	-	-21	-9	-8	0	2	3	4	5	7	10	42
Symptom scores												
Lower respiratory		←	Daily at home throughout study period →									
Upper respiratory		←	Daily at home throughout study period →									
ACQ-6	X	X	X		X							
Lung function												
Home PEFR		←	Daily at home throughout study period →									
Home FEV_1		←	Daily at home throughout study period →									
Clinic FEV_1	X	X	X		X	X	X	X	X	X	X	X
Clinic FEV_1:FVC ratio	X	X	X		X	X	X	X	X	X	X	X
Lung inflammation												
FeNO	X	X	X		X		X		X	X	X	X
PC_{20}	X	X	X						X			
Blood samples												
Blood eosinophils	X	X	X				X		X		X	X
Nasal samples		X	X		X	X	X	X	X	X	X	
Virus load		X	X		X	X	X	X	X	X	X	
Soluble mediators		X	X		X	X	X	X	X	X	X	
Bronchial samples												
Soluble mediators			X						X			
Flow cytometry												
Blood cell counts		X	X						X			X
Nasal cell counts		X	X						X			X
Bronchial cell counts			X						X			

4.10. Study Baseline

For Primary and Secondary endpoint assessments, baseline will be determined as the last measurement taken prior to virus inoculation i.e. Day 0 (ACQ-6, home PEFR, home FEV1, clinic FEV1, clinic FVC, FeNO, virus load, soluble mediators) where available. If/where this is missing, the value on the previous visit will be used, for example, laboratory bloods and PC20 (for laboratory bloods, the sample on day -8 will be taken as a baseline, for PC20, the result from day -9 will be taken).

Demographic baseline variables will be taken at Screening, Day -21 and Day 0 (where available).

5. Analysis Sets

5.1. Safety analysis set

The safety analysis set includes all subjects who were randomized and received at least one dose of study medication.

5.2. Full analysis set

The full analysis set (FAS) includes subjects who:

- Are randomised into the study (D-21)
- Have been inoculated with the rhinovirus serotype 16 (RV-16) challenge virus (D0)
- Have confirmed RV-16 infection, defined as either (i) positive RV-16 PCR in nasal lavage at any time after inoculation (D0) or (ii) seroconversion (positive antibodies to RV-16 at a titre of at least 1:4 at the final study visit)
- Have completed at least 14 days post inoculation with RV-16.

These subjects are defined as 'evaluable' and form the basis of the power calculation.

The FAS will be used to assess the primary objective and will be used to analyse all efficacy endpoints plus any post-infection mechanistic outcomes.

5.3. Per protocol analysis set

The per protocol (PP) analysis set includes subjects in the FAS without any major protocol deviations that could confound the interpretation of analyses conducted on the FAS. Major protocol deviations will be defined prior to database lock and without knowing the treatment of individual cases. Patients taking less than 80% of the prescribed medication will be excluded from the PP set. Patients that fail to provide a minimum 80% of endpoint data will also be excluded from per-protocol analysis from that particular endpoint.

Any per-protocol analysis would be limited to the primary and efficacy/symptom-based secondary endpoints as defined in Sections 6.2 & 6.3.1.

5.4. Extended analysis set

The extended analysis set expands on the FAS by including any subject who completed the study, regardless of whether they had confirmed RV-16 infection. The set will be used to investigate pre-infection mechanistic effects of OC459 and will also be used for any *ex-vivo* analyses.

6. Variables of Analysis

6.1. Baseline Demographic Variables

- Gender
- Ethnicity
- Age (at enrolment)
- Height, weight & BMI
- Duration of asthma
- History of previous oral steroids
- Skin prick test: (a) which allergens (b) cumulative wheal size in mm2
- Smoking history +/- pack years
- Baseline asthma medications (this includes the ICS dose)
- Values of the following at screening and, in supplementary tables, at randomisation (day -21) and at visit 4 (day 0):
    - ACQ-6
    - FEV_1 + reversibility
    - FVC
    - FeNO
    - PC_{20}
    - Blood eosinophils
    - Serum IgE
    - Serum vitamin D (screening and randomisation only)

6.2. Primary Analysis Variable

- The primary endpoint will consist of the sum of uncorrected lower respiratory symptom scores from day 0 to day 14. Daily scores will be out of 21 (seven symptom questions rated from zero to three) creating a potential maximum total score of 315.

6.3. Secondary Analysis Variables

6.3.1. Secondary Efficacy Variables

- Upper respiratory symptom score
- Lower respiratory symptom score
- ACQ-6 Score
- PEFR
- FEV_1

- FVC
- FeNO
- PC_{20}
- Virus load (via qPCR)

6.3.2. Secondary Mechanistic Variables

1. Blood & Sputum eosinophilia
2. Leukocyte numbers (e.g. ILC2, Th2) in BAL, blood and nasal scrapes measured by flow cytometry
3. Leukocyte numbers in immunohistochemistry-stained bronchial biopsies:
    - CRTH2+ cells (cells/mm2 tissue)
        - In the submucosa
        - In the epithelium
    - EG2+ activated eosinophils (cells/mm2 tissue)
        - In the submucosa
        - In the epithelium
4. Soluble Mediators (nasal and bronchial):
    - Prostaglandin D2
    - Th2 cytokines (3): IL-4, IL-5, IL-13
    - Th1 cytokines (2): IFN-γ, IL-12p70
    - Th17 cytokines (4): IL-17A, IL-17F, GM-CSF, IL-22
    - Chemokines (7): Eotaxin, Eotaxin 3, IP-10, MDC, TARC, MIP-1α, MIP-1β
    - Pro-inflammatory markers (4): IL-6, IL-8, IL-1β, TNF-α
    - Cytokines that can trigger type 2 inflammation (3): IL-25/-17E, IL-33, TSLP
    - Innate antiviral interferons (2): IFN-α, IL-29/IFN-λ1
    - Additional (6): IL-9, IL-10, IL-15, IL-18, IL-23, MIP-3α

6.3.3. Safety Analysis Variables

- Adverse events (recorded in free text; not coded)
- Serious adverse events
- Concomitant medications (recorded in free text, not coded)
- Vital signs (temperature, pulse, systolic/diastolic blood pressure, oxygen saturation levels, respiratory rate)
- Laboratory blood tests (full blood count, renal function, liver enzymes, clotting)

6.4. Exploratory Analysis Variables

Exploratory analysis will take place across all cell counts obtained above using flow cytometry and bronchial biopsy from blood, nasal and bronchoscopic samples: A comprehensive list of which can be found below:

- Total viable cells
- Total CRTH2+ cells

- Th2 cells (defined as CD3+CD4+CRTH2+)
  - Of which are also GATA3+
- Eosinophils (defined as CD66b+CD16-)
- Neutrophils (defined as CD66b+CD16+)
- Mast cells (defined as FcERI+Lin-CRTH2-CD117+)
- Basophils (defined as FcERI+Lin-CRTH2+CD117-)
- ILC2 cells (defined as Lin-CD127+FcERI-CRTH2+)
- ILC1 cells (defined as Lin-CD127+FcERI-CRTH2-CD117-)
- ILC3 cells (defined as Lin-CD127+FcERI-CRTH2-CD117+)

Blood samples will be assessed at day -21, day -8, day 5, day 42; nasal samples at day -21, day -8, day 5 and bronchoscopic samples at day -8, day 5 (counted by flow cytometry)

Further exploratory analysis will take place on cells obtained from bronchial biopsies from day -8 and day 5 by immunohistochemistry:

- CRTH2+ cells (cells/mm2 tissue)
  - In the submucosa
  - In the epithelium
- EG2+ activated eosinophils (cells/mm2 tissue)
  - In the submucosa
  - In the epithelium

Values for ACQ-6 (D-9, D10) will also be analysed.

In addition, exploratory analysis will take place to investigate antiviral response. This will consist of the following variables:

- Protein levels of IFN-β, -λ1 and –λ2/3 in supernatants of cells taken from subjects and infected with virus *ex vivo.*
- mRNA levels of IFN-β, -λ1 and –λ2/3 a panel of interferon stimulated genes (Mx1, RIG-I, OAS, viperin, IP-10, PKC) in cell lysates from the same *ex vivo* experiments.

7. Statistical Methodology

7.1. Baseline Demographics

Baseline demographic variables and other relevant clinical baseline characteristics as defined in Section 6.1 will be summarised for each treatment group.

Summaries of continuous variables will be presented as means and standard deviations if data is consistent with that from a normal population distribution, and as medians and inter-quartile ranges for data that is inconsistent. Categorical variables will be presented as frequencies and percentages.

7.2. Primary Analysis

Uncorrected lower respiratory symptom scores collected from D0 to D14 will be aggregated to create an overall total for each patient. Where patient scores are missing, data will be imputed based upon rules set within Section 7.5. Patients missing ≥20% of score data will be excluded from any additional per-protocol analyses.

Data will be presented using summary statistics per treatment group and will include median and interquartile range (IQR). A supplementary plot showing individual measurements for both groups with a box & whisker overlay showing median and IQR will be produced.

Primary analysis to test for difference between the mean aggregated scores of the two treatment groups will be carried out using Mann-Whitney U tests. Differences will be considered significant at $P < 0.05$. All P values are two-sided.

An additional secondary analysis will take place correcting the scores for bronchoscopy-related symptoms. Full methodology will be clarified within Section 7.3.1 below.

7.3. Secondary Analysis

7.3.1. Secondary Efficacy Analysis

Lower respiratory symptom scores collected from D0 to D14 will be corrected for the effects of bronchoscopy by subtracting the baseline symptoms value (mean average of values from D-13 to D-9) from the two days affected by bronchoscopy symptoms (day -8 and day -7). The adjusted residual symptom scores for day -8 and day -7 (which we assume to be purely bronchoscopy related) would then be subtracted from day 5 and day 6 to complete the correction.

Analysis to test for difference between the mean aggregated corrected scores of the two treatment groups will be carried out using Mann-Whitney U tests.

The effect of the drug on asthma exacerbations (as defined by change from day 0 to peak infection in symptoms, lung function, and measures of lung inflammation) will be using presented summary statistics per treatment group and will include median and interquartile range (IQR). Within-subject differences will be analysed using ANOVA and 2-tailed paired Student's t-tests or Wilcoxon signed rank test in the event where data is not consistent with a normal population distribution Analysis to determine the difference in effect of OC459 and placebo will be performed using unpaired (two-sample) t-test or Mann-Whitney U tests for data not consistent with a normal population distribution at each phase of the study.

Differences will be considered significant at $P < 0.05$ within a hierarchy of the primary outcome, and interpreted more cautiously for secondary efficacy outcomes, (likewise more so for

exploratory outcomes). Patients missing ≥20% of endpoint data will be excluded from any additional per-protocol analyses. Analyses that are conducted using the Per Protocol analysis set that are in agreement with conclusions derived from the Full analysis set will be more strongly interpreted than those in disagreement . All P values are two-sided.

7.3.2. Secondary Mechanistic Analysis

Soluble Mediators:

A total of 32 mediators in nasal samples (taken at day -21, 0, 2, 3, 4, 5, 7, 10) and bronchoscopic samples (taken at day -8, day 5) will be measured for each subject.

Values of soluble mediators obtained from bronchial samples will be presented using summary statistics per treatment group and visit. An additional table will summarise per treatment group (for each mediator) the difference in values between mediators taken at D-8 and D5. Analysis to investigate any difference in effect of OC459 and placebo for each mediator will be performed using the difference in values via unpaired (two-sample) t-test or Mann-Whitney U tests for non-parametric data.

Values of soluble mediators obtained from nasal samples will be presented using summary statistics per treatment group and visit. An additional table will summarize per treatment group (for each mediator) the difference between D-21 and peak value (the highest value measured between D0 and D10).

Taking the difference between D-21 and peak value, analysis to determine the difference in effect of OC459 and placebo for each mediator will be performed using unpaired (two-sample) t-test or Mann-Whitney U tests for non-parametric data.

Differences will be considered significant at $P < 0.05$ within a hierarchy of the primary outcome, and interpreted more cautiously for secondary efficacy outcomes, (likewise more so for exploratory outcomes). All P values are two-sided.

Leukocyte numbers:

Values of leukocyte numbers obtained from bronchial samples / flow cytometry will be presented using summary statistics per treatment group and visit. An additional table will summarise per treatment group (for each cell type) the difference in values between samples taken at D-8 and D5. Analysis to investigate any difference in effect of OC459 and placebo for each cell type will be performed using the difference in values via unpaired (two-sample) t-test or Mann-Whitney U tests for non-parametric data.

Eosinophil counts:

Values of blood and sputum eosinophilia will be presented using summary statistics per treatment group and visit. Analysis to investigate any difference in effect of OC459 and placebo for blood and sputum eosinophilia will be performed using the difference in values via unpaired (two-sample) t-test or Mann-Whitney U tests for non-parametric data.

7.3.3. Correlation testing between Secondary Outcomes

Potential causal relationships between outcome variables will be investigated using Spearman's rank correlations. Tests will be carried out between (but not limited to) the following:

Baseline values of:
• ACQ-6 (day 0)
• FeNO (day 0)
• Blood eosinophils (day -8)
• Serum IgE (day -8)
• PC_{20} (day -9)
• Skin prick test (from screening)
  ○ # of allergens
  ○ Sum of wheal diameters (mm)
• Soluble mediators
	vs	Peak and AUC values of:
		• Lower respiratory symptom score
		• Upper respiratory symptom score
		• Virus load
		• Morning PEFR (peak decline)
		• Morning FEV_1 (peak decline)
		• Soluble mediators

7.3.4. Safety Analysis

Adverse events will be summarised by treatment and severity. Separate tables summarising adverse events and their relationship to study treatment as well as summarising adverse events by treatment and timepoint will also be produced.

Serious adverse events will be listed and summarised by site, category and treatment. Separate tables will also be produced summarising serious adverse events and their relationship to study treatment as well as summarising serious adverse events by treatment and timepoint.

Vital Signs and Laboratory blood tests will be summarized by treatment per visit and will be presented as means and standard deviations if data is consistent with that from a normal population distribution, and as medians and inter-quartile ranges for data that is inconsistent. Categorical variables will be presented as frequencies and percentages.

If it is deemed appropriate, treatment effect will be assessed via t-test for continuous variables and Chi-square test or Fisher exact test for categorical variables (with the appropriate generalized linear model being used).

7.4. Exploratory Analysis

7.4.1. Exploratory Mechanistic Testing

Analysis to investigate any difference in effect of OC459 and placebo for the exploratory analyses below will be performed using the difference in values via unpaired (two-sample) t-test or Mann-Whitney U tests for non-parametric data.

- Changes in inflammatory cell counts by flow cytometry after drug treatment but before virus inoculation (for blood and nasal samples, not bronchial): Day -21 to Day -8.
- Changes in soluble mediators after drug treatment but before virus inoculation (nasal samples): Day -21 to Day 0.
- Changes in cell counts from bronchial biopsies taken at Day -8 and Day 5 will also be assessed CRTH2+ cells (cells/mm2 tissue) & EG2+ activated eosinophils (cells/mm2 tissue).

7.4.2. Exploratory Testing For Antiviral Response

Rhinovirus-induced IFN-β, λ1 and λ2/3 levels (protein and mRNA) will be measured in bronchial epithelial cell cultures stimulated with RV-16, RV-1B and Poly I:C after 6H, 24H and 48H. These will be compared across placebo and treatment groups, and to media and filtered virus stock controls.

Data will be presented in a summary table and will include mean and standard error. Statistical analysis will be carried out for multiple comparisons using ANOVA, or where non-parametric methods are required, Wilcoxon or Kruskal-Wallis testing. Differences will be considered significant at $P < 0.05$.

mRNA levels of a panel of interferon stimulated genes (MxA, RIG-I, OAS, viperin, IP-10, PKC) will also be analysed across the 3 timepoints using the same methods.

7.4.3. Exploratory Correlation Testing

We will correlate lower respiratory symptom scores with the ACQ (which reflects symptoms over the last 7 days) at two timepoints:

- ACQ-6 at day -9 vs sum of lower respiratory symptom scores day -15 to day -9 inclusive
- ACQ-6 at day 10 vs sum of lower respiratory symptom scores day 4 to day 10 inclusive

Potential relationships between the above will be investigated using Spearman's rank correlations.

Potential relationships between outcome variables and antiviral response variables will be investigated using Spearman's rank correlations. Tests will be carried out between the following:

Efficacy Analysis Variable • Lower respiratory symptom score (peak and AUC) • Upper respiratory symptom score (peak and AUC) • Virus load (peak and AUC) • Morning PEFR (peak decline) • Morning FEV_1 (peak decline) • FeNO (peak increase) • Any cytokine statistically induced by infection (peak increase)	vs	Antiviral Response Variable: • interferon levels (β, λ1, λ2/3) • mRNA levels of interferons (β, λ1, λ2/3) • mRNA levels of a panel of interferon-stimulated genes (MxA, RIG-I, OAS, viperin, IP-10, PKC)

7.4.4. Further Testing For Outcome Variables

In addition to the above exploratory tests, secondary outcome variables will be measured and analysed using alternative methods if deemed appropriate. Table 03 below contains a table showing the possible comparisons used:

Table 03: Potential Exploratory Analyses of Outcome Variables

	Comparison				
Variable:	Peak during infection (day 0 to 10)	Change from day 0 to peak infection	Area under curve during infection (day 0 to 10)	Total during infection (day 0 to 14)	Average value at each timepoint day 0 to 10
Lower respiratory symptom score[1]	X	X	N/A	X (primary outcome)	X
Upper respiratory symptom score[1]	X	X	N/A	X (secondary outcome)	X
Morning PEFR[2]	X (peak fall)	X (secondary outcome)	X	N/A	X
Morning FEV_1[2]	X (peak fall)	X (secondary outcome)	X	N/A	X
Clinic FEV_1[2]	X (peak fall)	X (secondary outcome)	X	N/A	X
ACQ-6	(day 10)	X (secondary outcome)	N/A	N/A	N/A
PC_{20}	(day 7)	X (secondary outcome)	N/A	N/A	(day -9 only)
FeNO	X	X (secondary outcome)	X	N/A	X
Blood eosinophils	X	X (secondary outcome)	N/A	N/A	X
Virus load	X (secondary outcome)	N/A	X	N/A	X
Soluble mediators - nasal[3]	X	X (secondary mechanistic outcome)	X	N/A	X
Soluble mediators - bronchial[3]	(day 5)	X (secondary mechanistic outcome)	N/A	N/A	(day -8 only)
Items marked as 'X' or with a specific study day are to be assessed as exploratory analyses					

7.5. Missing Data and Withdrawn Subjects

Missing values for primary and secondary endpoints (as per Table 03), viral load and soluble mediators will be extrapolated for cumulative / area under the curve calculations.

Imputation will be based upon the values before and after the missing timepoint(s). Missing values will be derived using the following formula:

$$x_n = x_{(n-1)} + z$$

Where:

x_n = missing value at day 'n'

$x_{(n-1)}$ = last recorded value before missing data begins

z is a constant derived from the last recorded value $x_{(n-1)}$, the first value obtained after missing data y, and d = the number of missing data points between $x_{(n-1)}$ & y

$$z = \frac{y - x_{(n-1)}}{d + 1}$$

For example; if we are missing data at day 4 and we know that the value at day 3 is 1 and the value at day 5 is 3 then we can calculate constant z as $z = \frac{(3-1)}{(1+1)} = 1$ thus the missing value at day 4 would be: $x_4 = (x_3 + 1) = (1 + 1) = 2$.

Imputation and analysis involving missing and imputed data will be taken under the assumption that the data is missing-at-random (MAR).

If imputation has taken place, sensitivity analyses will be performed to ensure integrity of statistical testing based on extrapolated scores.

No other values for missing data will be imputed.

If there is a missing baseline value for virus load, the previous value (i.e. at randomisation / day -21) will be taken (virus load should be below the lower limit of detection prior to inoculation). If there is a missing baseline value on another variable and after unblinding the subject is in the placebo group, the baseline will be replaced with a value from a previous visit (e.g. randomisation / day -21) if available. If after unblinding the subject is in the treatment group, the baseline will not be replaced (with the exception of virus load, as above).

7.6. Data Handling & Transformation

ACQ scores will be calculated under the ACQ-6 variant of the form. The score will be derived by taking the mean average of the 6 values provided (note that whilst FEV is recorded this will not formulate part of the overall score).

Viral loads below the limit of quantification will be treated as zero. Viral load data will be transformed using base 10 logarithm. To account for zero values, one will be added to each viral load measurement before being transformed.

Soluble mediators which generate a detectable signal but are below the lower limit of detection will have an assumed value of half the lower limit of detection; those for which no signal was detected will have an assumed value of zero. Soluble mediators which are above the upper limit of detection will have an assumed value of the highest reading of all the samples on the same assay performed at that time. Normal transformation and/or sensitivity analyses may be performed as necessary depending on the data and the extent of values above and below limits of detection.

AUC values for exploratory analyses will be calculated using the linear trapezoidal method. Missing values will be extrapolated where required.

7.7. Software of Analysis

Statistical analysis of primary and secondary endpoints will be performed using SAS 9.4.
Additional graphical output may be performed using R.

APPENDIX A – INDEX OF TABLES, FIGURES & LISTINGS

Baseline Variables

Primary Analysis

Secondary Efficacy Analysis

Secondary Mechanistic Analysis

APPENDIX B – SHELL TABLES, FIGURES & LISTINGS

Baseline Variables

Table 1.01 – Summary of Demographic Baseline Variables

Variable*	Statistics	OC459 (N = xx)	Placebo (N = xx)
Age (y)	N		
	Mean		
	SD		
	Min		
	Median		
	Max		

*Variables for inclusion as per section 6.1

Table 1.02 – Summary of Demographic Variables at Randomisation (Day -21)

See Table 1.01

Table 1.03 – Summary of Demographic Variables at Innoculation (Day 0)

See Table 1.01

Primary Analysis

Table 2.01 – Listing of Lower Respiratory System Scores

		Visit Scores															
Treatment	**Subject**	**D0**	**D1**	**D2**	**D3**	**D4**	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**	**D11**	**D12**	**D13**	**D14**	**Total**

Table 2.02 – Summary of Uncorrected Total Lower Respiratory System Scores

Treatment	**Visit**	**n**	**Mean**	**SD**	**Median**	**Min.**	**Max.**	**IQR**
OC459	D0							
	…							
	D14							
	Total							
Placebo	D0							
	…							
	D14							
	Total							

Table 2.03 – Mann-Whitney U Test of Total Lower Respiratory System Scores

Median Scores (Number of Points Above Median) for Variable Response Classified by Variable Treatment					
Treatment	N	Sum of Scores	Expected Under H0	Std Dev Under H0	Mean Score
Active	xx	xx	xx	xx	xx
Placebo	xx	xx	xx	xx	xx
Average scores were used for ties.					

Two-Sample Test	
Statistic (S)	xx
Normal Approximation	
Z	xx
One-Sided Pr > Z	xx
Two-Sided Pr > |Z|	xx
t Approximation	
One-Sided Pr > Z	xx
Two-Sided Pr > |Z|	xx
Exact Test	
One-Sided Pr >= S	xx
Two-Sided Pr >= |S - Mean|	xx

Figure 2.04 – Box & Whisker Plot of Total Lower Respiratory System Scores

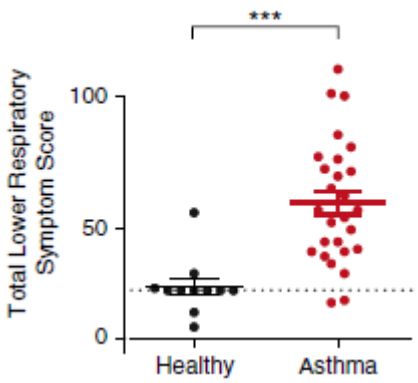
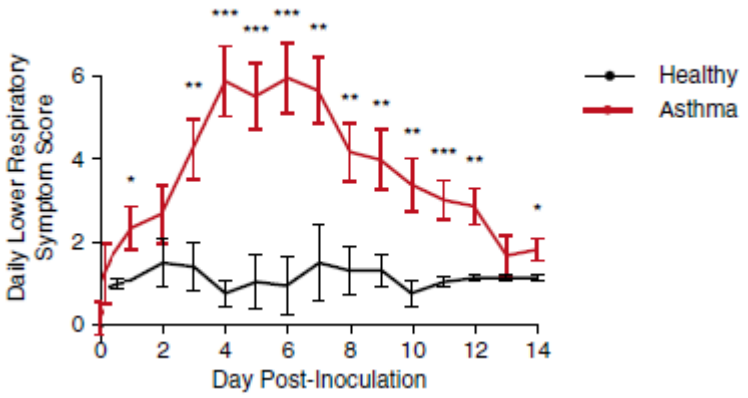


Figure 2.05 – Box & Whisker Plot of Daily Lower Respiratory System Scores



Secondary Efficacy Analysis

Table 3.01 – Summary of Corrected Total Lower Respiratory System Scores

See Table 2.02

Table 3.02 – Mann-Whitney U Test of Corrected Lower Respiratory System Scores

See Table 2.03

Table 3.03 – Summary of Total Upper Respiratory System Scores

See Table 2.02

Table 3.04 – Mann-Whitney U Test of Total Upper Respiratory System Scores

See Table 2.03

Table 3.05 – Summary of Effect of Treatment on Asthma Exacerbation Variables

Laboratory Test (units)*	Treatment	Visit	n	Mean	SD	Median	Min.	Max.	IQR
xxxxx (xxxx)	Control								
	Treatment								

*Variables for inclusion as per section 6.3.1

Table 3.06 – Statistical Testing on Effect of Treatment on Asthma Exacerbation Variables

For normally distributed data variables:

Source	DF	Sum of Squares	Mean Square	F Value	Pf > F
Model	xx	xx	xx	xx	xx
Error	xx	xx	xx		
Corrected Total	xx	xx			

For non-parametric data variables: See Table 2.03

Table 3.07 – Summary of Effect of Treatment on Viral Load Results

Laboratory Test (units)*	Treatment	Visit	n	Mean	SD	Median	Min.	Max.	IQR
xxxxx (xxxx)	Control								
	Treatment								

*Variables for inclusion as per section 6.3.1

Table 3.08 – Statistical Testing on Effect of Treatment Viral Load Results

For normally distributed data variables:

Source	DF	Sum of Squares	Mean Square	F Value	Pf > F
Model	xx	xx	xx	xx	xx
Error	xx	xx	xx		
Corrected Total	xx	xx			

For non-parametric data variables: See Table 2.03

Secondary Mechanistic Analysis

Table 3.09 - Summary of Nasal Soluble Mediators per visit

Laboratory Test (units)*	Treatment	Visit	n	Mean	SD	Median	Min.	Max.	IQR
xxxxx (xxxx)	Control								
	Treatment								

Table 3.10 - Summary of Change from Baseline to Peak Value for Nasal Soluble Mediators

See Table 3.09

Table 3.11 - Statistical Testing of Nasal Soluble Mediators

For normally distributed data variables:

Source	DF	Sum of Squares	Mean Square	F Value	Pf > F
Model	xx	xx	xx	xx	xx
Error	xx	xx	xx		
Corrected Total	xx	xx			

For non-parametric data variables: See Table 2.03

Table 3.12 - Summary of Bronchial Soluble Mediators per visit (D-8, D5)

See Table 3.09

Table 3.13 - Summary of Change from Baseline (D-8) to Infection (D5) for Bronchial Soluble Mediators

See Table 3.09

Table 3.14 - Statistical Testing of Bronchial Soluble Mediators

See Table 3.11

Table 3.15 - Summary of Leukocyte Numbers per visit (D-8, D5)

See Table 3.09

Table 3.16 - Summary of Change from Baseline (D-8) to Infection (D5) for Leukocyte Numbers

See Table 3.09

Table 3.17 - Statistical Testing of Leukocyte Numbers

See Table 3.11

Table 3.18 - Summary of Blood and Sputum Eosinophilia

See Table 3.09

Table 3.19 - Summary of Change from Baseline to Peak Value for Blood and Sputum Eosinophilia

See Table 3.09

Table 3.20 - Statistical Testing for Blood and Sputum Eosinophilia

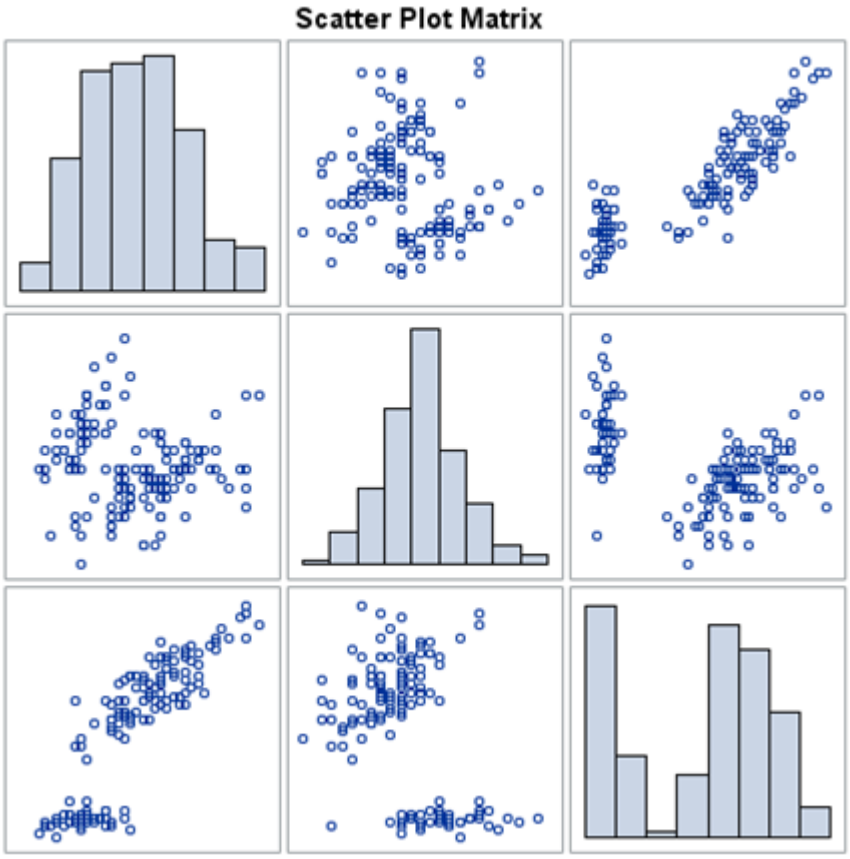
See Table 3.11

Secondary Analysis – Correlation Testing

Table 3.21 - Spearmans Rank Correlation Testing

Pearson Correlation Coefficients Prob > |r| under H0: Rho=0 Number of Observations		
	Var 1	Var 2
Var	xxxx	xxxx
xxxxxxx.	xxxx	xxxx
	xx	xx
Var	xxxx	xxxx
xxxxxxx.	xxxx	xxxx
	xx	xx

Figure 3.22 - Correlation Matrix



The above matrix can contain the following:

Baseline values of:

- ACQ (day 0)
- FeNO (day 0)
- Blood eosinophils (day -8)
- Serum IgE (day -8)
- PC_{20} (day -9)
- Skin prick test (from screening)
  - # of allergens
  - Sum of wheal diameters (mm)
- Soluble mediators

vs

Peak and AUC values of:

- Lower respiratory symptom score
- Upper respiratory symptom score
- Virus load
- Morning PEFR (peak decline)
- Morning FEV_1 (peak decline)
- Soluble mediators

Secondary Safety Analysis

Table 3.23 - Summary of Adverse Events by Treatment and Timepoint

		Events			Patients		
		OC459	Placebo	Total	OC459	Placebo	Total
Timepoint	**Total Number of AEs**						
	Pre-Treatment (Wk1)						
	Pre-Inoculation (Wk2)						
	Assessment Phase (Wk3-4)						
	Follow-Up (Wk5-9)						

Table 3.24 - Summary of Adverse Events by Treatment and Severity

		Events			Patients		
		OC459	Placebo	Total	OC459	Placebo	Total
Severity	**Number of Adverse events**						
	Mild						
	Moderate						
	Severe						
Reason for SAE	**Number of SAEs**						
	Death						
	Life threatening						
	…						
	Other medical important events						
	Total						

Table 3.25 - Summary of Adverse Events by Treatment and Relationship to Study Treatment

	All Events						
Treatment	**None**	**Unlikely**	**Possible**	**Probable**	**Definitely**	**Not Yet Defined**	**Total**
OC459							
Placebo							
All subjects							
	Patients with AEs*						
Treatment	**None**	**Unlikely**	**Possible**	**Probable**	**Definitely**	**Not Yet Defined**	**Total**
OC459							
Placebo							
All subjects							

***Table Note: Where subjects have more than one AE the highest relationship has been used.**

Table 3.26 - Listing of Serious Adverse Events

OC459:

Subj.	AE Diagnosis	Details	Relation to Treatment	Start Date	Ongoing /End Date	Duration	Expected-ness	Severity

Placebo:

Subj.	AE Diagnosis	Details	Relation to Treatment	Start Date	Ongoing /End Date	Duration	Expected-ness	Severity

Table 3.27 - Summary of Serious Adverse Events by Treatment and Timepoint

		Events			Patients		
		OC459	Placebo	Total	OC459	Placebo	Total
Timepoint	**Total Number of SAEs**						
	Pre-Treatment (Wk1)						
	Pre-Inoculation (Wk2)						
	Assessment Phase (Wk3-4)						
	Follow-Up (Wk5-9)						

Table 3.28 - Summary of Serious Adverse Events by Treatment and Severity

		Events			Patients		
		OC459	Placebo	Total	OC459	Placebo	Total
Severity	Number of Serious Adverse events						
	Mild						
	Moderate						
	Severe						

Table 3.29 - Summary of Serious Adverse Events by Treatment and Relationship to Study Treatment

	All Serious Adverse Events						
Treatment	None	Unlikely	Possible	Probable	Definitely	Not Yet Defined	Total
OC459							
Placebo							
All subjects							
	Patients with SAEs*						
Treatment	None	Unlikely	Possible	Probable	Definitely	Not Yet Defined	Total
OC459							
Placebo							
All subjects							

***Table Note: Where subjects have more than one SAE the highest relationship has been used.**

Table 3.30 - Summary of Vital Signs Results

Laboratory Test (units)*	Treatment	Visit	n	Mean	SD	Median	Min.	Max.
xxxxx (xxxx)	Control							
	Treatment							

*Variables for inclusion as per section 6.3.4

Table 3.31 - Summary of Clinical Chemistry & Haematology Results

Laboratory Test (units)*	Treatment	Visit	n	Mean	SD	Median	Min.	Max.
xxxxx (xxxx)	Control							
	Treatment							

*Variables for inclusion as per section 6.3.4

Exploratory Analysis

Table 4.xx - Exploratory Analyses as required

Extent of output determined by Section 7.4