Clinical Trial Phase I

The effects of OC000459 (a CRTH2 antagonist) on nasal Th2 cytokine (IL-4/5/13) release, eosinophil responses, and nasal symptoms after nasal allergen challenge (NAC) with Timothy grass pollen in subjects with allergic rhinitis out of season: A double blind, randomised, two way crossover, placebo-controlled study in up to 20 subjects of the effect of a short course of OC000459 (400mg oral, OD with food am for 5 days)

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Chief Investigator: Trevor Hansel
Medical Director
National Heart and Lung Institute Clinical Studies Unit,
First Floor, Royal Brompton Hospital,
Fulham Road,
London SW3 6HP,
United Kingdom.
Telephone: 020 7351 8974
Facsimile: 020 7351 8973
Email: t.hansel@imperial.ac.uk

Study Sponsor: Oxagen Ltd.,
91 Milton Park,
Abingdon,
OXON,
OX14 4RY,
United Kingdom.
Authorised representative: Dr C Mike Perkins.
Telephone: 01235 443300.
Facsimile: 01235 443301.
Email: m.perkins@oxagen.co.uk

Medical Monitor: Dr J Steiner,
Oxford Therapeutics Consulting Ltd.,
Magdalen Centre,
The Oxford Science Park,
Oxford OX4 4GA,
United Kingdom.
Telephone: 01865 784874.
Facsimile: 01865 784875.
Mobile: 07774 967074.

Clinical Research Organisation:
Akos Limited,
The Coach House,
The Grove,
Piper's Lane,
Harpenden
Hertfordshire AL5 1AH
Telephone: 01582 766339
Facsimile: 01582 764327

Protocol amendment page

List dates of amendments and attach text if not incorporated into protocol.

Approval signature page

Approved by:

C Mike Perkins MD, Director of Development
Oxagen Ltd

Date 9 January 2006

Jan Steiner, Medical Monitor
Oxford Therapeutics Consulting

Date 8 Jan 2006

Nicola McKelvie, Regulatory Affairs
Origin Pharmaceutical Services Ltd

Date 3 Jan 2006

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LIST OF ABBREVIATIONS

°C	Degrees centigrade
µL	Microlitres
µm	Micromolar
ABPI	Association of the British Pharmaceutical Industry
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
a.m.	In the morning (Ante meridian)
AST	Aspartate aminotransferase
AUC	Area under the curve
BSA	Bovine serum albumen
BU	Biological units
COREC	Central Office for Research Ethics Committees
CRF	Case report form
CRThH2	Chemoattractant receptor expressed on ThH2 cells
CV	Coefficient of variation
EC	European Community
ECG	Electrocardiogram
ELISA	Enzyme linked immunosorbent assay.
FEV1	Forced expiratory volume over one second
g	Grams
g	Gravitational constant
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GM-CSF	Granulocyte macrophage colony stimulating factor
hr	Hour(s)
ICH	International Conference on Harmonisation
IL	Interleukin
Kg	Kilogram
LC-MS/MS	Liquid chromatography/mass spectrometry
m	Metre
MCHC	Mean corpuscular haemoglobin concentration
MCV	Mean corpuscular volume
mg	Milligram
MHRA	Medicines and Healthcare products Regulatory Agency
min	Minute
MIP	Macrophage inhibitory protein
ml	Millilitre
mm	Millimetre
Main REC	Main Research Ethics Committee
mRNA	Messenger ribonucleic acid
n	Number
NAC	Nasal allergen challenge
NCR	No carbon required
NHLI	National Heart and Lung Institute
OD	Once daily
PBS	Phosphate buffered saline
PGD_2	Prostaglandin D2
PK	Pharmacokinetic
p.m.	In the afternoon (post meridian)
RANTES	Regulated on activation, normal T expressed and secreted
rpm	Revolutions per minute

SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SUSAR	Suspected unexpected serious adverse reaction
Th	T-helper
TNF	Tumour necrosis factor
U.K.	United Kingdom
WHO	World Health Organisation

SUMMARY/SYNOPSIS

Objectives

Primary

To assess the effects of OC000459 on levels of IL-4, IL-5 and IL-13 (Th2 cytokines) in nasal filter paper (measured using Luminex) for 1 to 9hr after nasal allergen challenge.

Secondary

To assess the effects of OC000459 on the following parameters after nasal allergen challenge as follows:

- Th2 cytokines at 15 and 30min after nasal allergen challenge.
- Total eosinophil counts (number of eosinophils/ml) in nasal lavage fluid after nasal allergen challenge.
- PGD_2 levels in nasal filter paper after nasal allergen challenge.
- Total nasal symptoms scores after nasal allergen challenge.

To assess the safety of OC000459 in terms of adverse events, vital signs and safety laboratory parameters.

To assess plasma concentrations of OC000459 after five doses of 400mg daily and their relation to inhibition of Th2 cytokines, PGD_2 and eosinophils in nasal fluid after allergen challenge.

Study design

The study will be a randomised, double blind, placebo controlled, two way crossover evaluation of the effect of OC000459 on cytokine secretion induced by nasal allergen challenge with Timothy grass extract. Up to 20 (to yield 16 evaluable) male subjects with a known history of grass pollen related allergic rhinitis and screening positive to allergen challenge with Timothy grass extract by skin prick test and nasal allergen challenge with Timothy grass extract will be included. After five days of dosing with OC000459 400mg or placebo, nasal allergen challenge will be performed and the following will be measured in the nasal fluids: IL-4, IL-5, IL-13 (primary endpoints, nasal filter paper), PGD_2 (nasal filter paper) and eosinophils (in nasal lavage washings). Nasal symptom scores will also be recorded. There will be a 14-21 day washout period between the first and second treatment periods of the study (Treatment Periods 1 and 2). Safety parameters will be monitored throughout and blood for drug plasma concentrations will be drawn before and after dosing with test article.

Study population

Inclusion criteria

1. Males aged 18 to 50 years with a history of symptoms of grass pollen related allergic rhinitis within the previous two years.
2. Subjects must be free from significant cardiac, pulmonary, gastrointestinal, hepatic, renal, haematological, neurological and psychiatric disease as determined by history, physical examination and screening investigations.
3. FEV_1 within normal limits.

4. Atopy defined by a positive cutaneous response (wheal ≥ 3mm compared to control) to Timothy grass pollen.
5. Asymptomatic at screening as characterized by:
    a. Normal appearing nasal mucosa with no active allergic rhinitis.
    b. A total nasal symptom score sheet on study entry so that subjects produce a score of <2 at screening and for 3 days before treatment periods 1 and 2.
6. Non smokers for at least the past 12 months with a pack history ≤ 1 pack years (Pack years = (No of cigarettes smoked/day/20) x No of years smoked).
7. An eosinophilic nasal response after NAC with Timothy grass pollen. Following the NAC and filter paper collection noted above, a nasal lavage will be performed in each nostril at 1hr. Levels of eosinophils must be ≥20,000 eosinophils/ml of nasal lavage fluid in either nostril.
8. Capable of giving informed consent, which includes compliance with the requirements and restrictions listed in the consent form.
9. Available to complete the study.
10. Subjects with a negative urinary drugs of abuse screen, determined within 14 days of the start of the study, and throughout the study treatment periods.
11. Negative carbon monoxide test (smokerlyzer) determined within 14 days of treatment period 1, and throughout the study treatment periods.
12. Subjects with a normal 12-lead electrocardiogram (ECG), determined at screening.

Exclusion criteria

1. Medical conditions likely to affect the outcome of the study.
2. Nasal conditions likely to affect the outcome of the study, i.e. nasal septal perforations, nasal polyps, sinus disease, chronic nasal obstruction, or other nasal diseases.
3. Presence of any respiratory disease other than a history of mild stable asthma not requiring treatment and associated with normal lung function (defined as ≥ 90% predicted for height and age).
4. History of immunotherapy in the past 3 years or currently on an immunotherapy treatment course including inhaled or local corticosteroids in the past 28 days.
5. Any infirmity, disability, or geographic location which, in the opinion of the principal investigator, would limit compliance with the protocol.
6. Infection of the upper airways/lower airways, sinus, or ear, including viral infections in the 14 days prior to screening and at the start of each treatment period.
7. Clinically significant abnormality in clinical laboratory tests at screening as determined by the principal investigator.
8. Inability to tolerate lavage correctly with a preliminary nasal lavage at screening.
9. The subject has participated in a study with a new molecular entity during the previous four months or any other trial during the previous three months.
10. The subject regularly, or on average, drinks more than four units of alcohol per day.
11. The subject has a history of testing positive for hepatitis C antibody or hepatitis B surface antigen or for HIV.
12. A history of gastrointestinal disorder likely to influence drug absorption.
13. Receipt of prescribed or over the counter medication within 14 days of the first study day and for the duration of the trial, including vitamins. In particular, all non-steroidal anti-inflammatory drugs (e.g. ibuprofen) are prohibited during this period.

14. Inability to communicate well with the investigator (i.e., language problem, poor mental development or impaired cerebral function).
15. Donation of 450 ml or more blood within the previous 12 weeks.
16. A history of hypersensitivity and/or idiosyncrasy to any of the test compounds or excipients employed in this study.
17. Any previous clinical trial involving the administration of OC000459.
18. Subjects with clinical features suspicious of tuberculosis – weight loss, pyrexia, haemoptysis, purulent sputum, abnormal chest X-ray will be excluded from the study.
19. Unwilling to eat set breakfast.
20. Unwilling to use a reliable form of contraception during the study and for a week after the last exposure to test article.

Withdrawal criteria

1. Subject experiences a serious or intolerable adverse event that prevents him from continuing.
2. Subject incurs a significant protocol violation.
3. Subject requests early discontinuation.
4. Request of the Sponsor.
5. Investigator's request (e.g. if the Investigator considers that the subject's health is compromised by remaining in the study or the subject is not sufficiently co-operative).
6. Subject is lost to follow-up.

Treatment plan and methods

Male healthy volunteers conforming to the selection criteria will be invited to take part in the study. After giving fully informed written consent, they will undergo a complete screening assessment. This screening assessment will not take place more than two weeks prior to the start of dosing or less than seven days.

Subjects will commence dosing with OC000459 400 mg or placebo on day 1 (visit 2 or visit 5) of each treatment period of the study. Blood will be drawn for safety testing (haematology and clinical chemistry) and urine will be obtained for a screen for drugs of abuse. The urine will also be subjected to dipstick analysis for protein, blood, ketones and urobilinogen. A smokerlyzer test will be performed along with an alcohol breathalyser test. Adverse events and concomitant medications will be noted in the CRF and dosing with the test article will then be done under supervision. Subjects will be given further supplies to take home. Subjects should take each dose (4 capsules in the morning) as soon as they have finished breakfast.

On day 4 of the study period (visit 3 or visit 6), subjects will report to the Clinical Unit at 21.00 hours. Adverse events and concomitant medications will be noted in the CRF for the period since the day 1 visit. A smokerlyzer test will be performed along with an alcohol breathalyser test. A urine sample will also be tested for drugs of abuse. A positive result on any of these three tests (smoking, alcohol, drugs) will make the subject ineligible to continue in the study. On the following morning (day 5), blood will be drawn for safety laboratory testing and plasma drug concentration. A urine sample will be tested by dipstick analysis for protein, blood, ketones and urobilinogen. Vital signs (Supine and standing blood pressure and pulse rate, respiration rate, oral temperature) will be recorded. A high fat breakfast will be served at about 6.30 a.m. The fifth dose of test article will then be taken at 7.00 a.m.

Two hours and thirty minutes after **dosing** a total nasal symptom score will be documented and a nasal lavage will be carried out on both nostrils and discarded.

At two hours and forty five minutes after dosing a single filter paper will be taken from the right nostril and nasal lavage will be undertaken in the left nostril. Two hours and fifty five minutes after dosing, blood will be drawn for plasma drug level and blood pressure and heart rate will be recorded. Three hours after **dosing**, a nasal allergen challenge in both nostrils using Timothy grass extract will be carried out.

Following the **nasal allergen challenge**, the following tests will be carried out:

Nasal symptom score at: 30min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, 9hr.

Nasal filter paper (right nostril only) at: 15min, 30min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, 9hr.

Nasal lavage (left nostril only) at: 30min, 1hr, 2hr, 4hr, 6hr, 8hr.

Blood will be drawn for measurement of plasma concentrations of test article at: 2hr, 4hr, 6hr, 8hr.

Heart rate and blood pressure will be recorded at the following times: 2hr, 4hr, 6hr, 8hr.

Adverse events and concomitant medications will be recorded throughout the study period.

Finally, sampling for laboratory safety tests (urine dipstick analysis for protein, blood, ketones and urobilinogen and blood for haematology and clinical chemistry) will be carried out at least 12 hours after dosing.

Following completion of all study related procedures that day, subjects may go home unless they have suffered an adverse event that requires further hospital treatment.

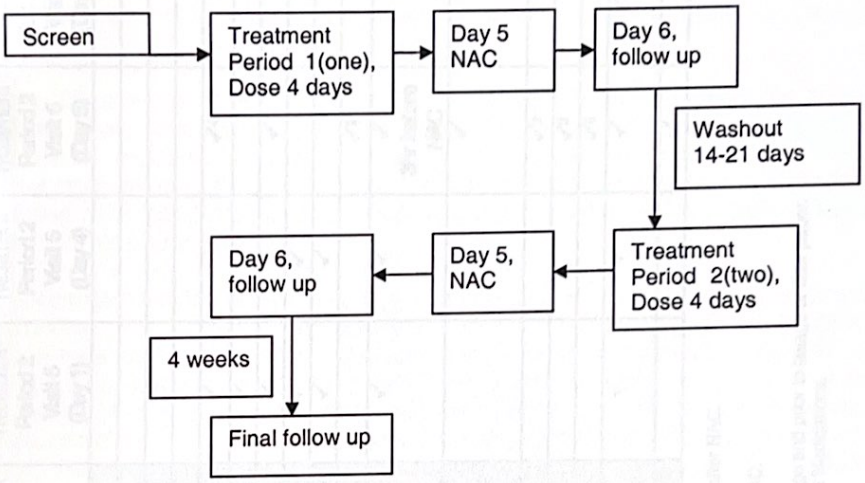
They will be contacted by a member of staff from the Clinical Studies Unit the following morning (Visit 4 and Visit 7) to document any new adverse events or changes in concomitant medications.

The second treatment period (Treatment Period 2) of the study will commence 14-21 days after the final blood samples in the first treatment period (Treatment Period 1) have been drawn and will be identical to the first treatment period (Treatment Period 1) of the study.

A final follow-up visit will be conducted four weeks after the last dose of test article in Treatment Period 2.

Total blood loss for this study is expected to be approximately 120 ml.

Study schedule



Flow chart

Procedure/Visit	Screen Visit 1	Treatment Period 1 Visit 2 (Day 1)	Treatment Period 1 Visit 3 (Day 4)	Treatment Period 1 Visit 3 (Day 5)	Treatment Period 1 Visit 4 (Day 6)	Washout Period 14-21 days	Treatment Period 2 Visit 5 (Day 1)	Treatment Period 2 Visit 6 (Day 4)	Treatment Period 2 Visit 6 (Day 5)	Treatment Period 2 Visit 7 (Day 6)	Follow-up Visit 8
Informed consent	✓										
Medical history	✓										✓
Physical examination	✓										
Skin prick test	✓										✓
Vital signs	✓	✓		✓[4]			✓		✓[4]		✓
12-lead ECG	✓						✓				✓
Laboratory safety samples	✓	✓		✓			✓		✓		✓
Alcohol and smokerlyzer test	✓	✓	✓				✓	✓			
Urine drug screen	✓	✓	✓				✓	✓			
Plasma drug concentrations				✓[5]					✓[5]		
Dosing: OD, am for 5 days		✓	✓	✓ 3hr before NAC			✓	✓	✓ 3hr before NAC		
Nasal allergen challenge (NAC) Timothy grass pollen 500BU / nostril	✓			✓					✓		
Nasal filter paper				✓[2]					✓[2]		
Nasal lavage	✓[1]			✓[3]					✓[3]		
Total nasal symptoms scores	✓			✓[6]					✓[6]		
Adverse event & Concomitant medication queries	✓	✓	✓	✓	✓[7]		✓	✓	✓	✓[7]	✓
Study medication reconciliation				✓					✓		

[1] A single nasal lavage in both nostrils at 1h after NAC.
[2] Nasal filter paper to be carried out at 15min before and then 15min, 30min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr ,7hr, 8hr and 9hr after NAC.
[3] Nasal lavage to be carried out at 15min before and then 30min, 1hr, 2hr, 4hr, 6hr and 8hr after NAC.
[4] Heart rate and blood pressure to be measured pre-dose, pre nasal allergen challenge and 2hr, 4hr, 6hr and 8hr after NAC.
[5] Blood to be drawn pre-dose, pre allergen challenge and 2hr, 4hr, 6hr and 8hr after challenge.
[6] Nasal symptom scores to be recorded before and 30 min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, 9hr after allergen challenge and prior to lavage or filter paper.
[7] Subjects will be contacted by phone the following morning to query any new Adverse Events or changes in Concomitant Medications.

1. INTRODUCTION AND RATIONALE

CRTH2 (Chemoattractant Receptor expressed on Th2 cells) is a G protein-coupled receptor expressed by Th2 lymphocytes, eosinophils and basophils and mediates chemotaxis of these cells in response to prostaglandin D_2 (PGD_2), the major prostanoid produced by mast cells.

OC000459 is a potent and selective CRTH2 antagonist which blocks the ability of PGD_2 to cause chemotaxis and activation of Th2 lymphocytes and eosinophils and therefore is expected to suppress airway inflammation associated with asthma. Studies performed *in vitro* and *in vivo* indicate that this mechanism is important in mediating mast cell-dependent accumulation and activation of Th2 lymphocytes and eosinophils as occurs in asthma.

It is anticipated that OC000459 will be a once-a-day oral product that will be effective in reducing the airway inflammation in mild to moderate asthma leading to improvement in lung function. It is unlikely to have bronchodilator activity and, although it will have a delayed onset of action, it should improve lung function more effectively than the leukotriene antagonist, Singulair™, but with equivalent safety. It is anticipated that the drug will be used in mild-to-moderate asthmatics and as an add-on therapy to those patients whose disease is not adequately controlled by steroids alone.

OC000459 has been administered to 30 healthy male volunteers in doses of 10mg, 25mg, 50mg, 10 mg once daily and 100mg twice daily. All dose levels were well tolerated and only two possible adverse reactions were recorded, both of them mild. No clinically significant changes in vital signs or laboratory abnormalities occurred. Predictable pharmacokinetics were observed when administered in the fasting state. However, after a standardised full fat meal, there appeared to be up to a fourfold increase in bioavailability. In a subsequent multiple dose study, doses of 100mg, 200mg and 400mg were studied for 7 or 14 days' duration. All dose levels were well tolerated and the pharmacokinetic profiles were linear in relation to dose. This second study confirmed the increase in oral bioavailability induced by food.

<u>Nasal allergen challenge tests.</u>

The nose is much more accessible than the airways to assess the effects of anti-inflammatory therapy. It is possible to obtain repeated samples of nasal exudates and mucosa cells before and after nasal allergen challenge (NAC) in a relatively non-invasive way by techniques such as nasal lavage, filter paper, and nasal brushing and scraping. A comprehensive review of the extensive clinical research experience with these nasal methodologies has recently been published by Peter Howarth and colleagues[1]. Nasal samples can then be analysed by novel semi-automated analytical methods to assess chemokines and cytokines, inflammatory mediators, mRNA and transcription factors. The other challenge test commonly used to investigate efficacy of asthma therapies is the inhaled allergen challenge which produces a significant decrease in FEV_1, while the nasal symptoms that follow NAC are generally mild. Furthermore, it is much easier to recruit potential subjects with allergic rhinitis due to grass pollen outside the hay fever season than asthmatic subjects with a dual early and late reaction to an inhaled allergen. Following NAC it is possible to measure symptoms, employ acoustic rhinomanometry and measure levels of cells and mediators to evaluate new drugs for allergic rhinitis and asthma[2-4].

It has long been recognised that there is a strong functional and immunological relationship between the nose and bronchi[5,6], especially in terms of infiltrating leukocytes and inflammatory mediators when comparing allergic rhinitis and allergic

asthma[7]. The upper and lower airways have related respiratory epithelium and similar responses to allergen challenge. Indeed, allergic rhinitis and asthma commonly coexist[8], since allergy is a systemic disorder that can affect various organs within the unified immune system[9,10]. This is in line with the WHO Initiative on Allergic Rhinitis and its Impact on Asthma (ARIA) stressing the concept of a single airway disease[11]. However, the nasal model involves a different vasculature to that in the airways, while the bronchi have added airway smooth muscle. At a pathological level, the extent of nasal remodelling in allergic rhinitis seems to be much less than that in the bronchi of asthmatic patients[12,13].

There is strong evidence that allergen-reactive type 2 T helper (Th2) cells play an important role in the induction and maintenance of the allergic inflammatory cascade[14]. Cytokines and chemokines produced by Th2 cells (IL-4, IL-5, IL-6, and IL-13) may be pivotal to the pathophysiology of allergic disorders: involving production of IgE; recruitment and activation of mast cells and eosinophils; mucus hypersecretion; subepithelial fibrosis; and tissue remodelling. Several studies have demonstrated significant expression of various cytokines and chemokines in inflammatory cells at sites of nasal allergic inflammation[15-19]. Excessive production of IL-5 and IL-13, may be critical to the allergic response[14,20].

Maintenance treatment with topical steroids exerts a range of anti-inflammatory nasal effects on production of eotaxin[21], RANTES, MIP-1α, IL-8, IL-1β[17], TNF-α[22] and IL-5[23]. Topical allergen challenge increases the levels of mucosal mRNA of IL-5 and IL-13[19,23] but nasal cytokines and chemokines may be produced at low concentration in nasal secretions, and may be undetectable when employing conventional ELISAs. A single dose of topical corticosteroid has been shown to reduce levels of GM-CSF and IL-5 detected by absorption with filter paper following nasal challenge with grass pollen in allergic rhinitis[23,24].

In order to sample nasal exudates for allergic inflammatory mediators, the classical methods of nasal lavage are those described by Naclerio et al.[25], the nasal pool method of Greiff et al.[26], and the use of a Foley's catheter by Grünberg and colleagues[27]. Lavage is performed with saline with volumes between 1 and 10ml of lavage fluid. The repeatability and validity of different nasal lavage methods have been compared[28]. An important demonstration of the utility of this methodology for assessing therapy was the demonstration that pre-treatment with topical corticosteroids causes inhibition of release of histamine, kinins and symptoms after NAC[29]. Peptidyl leukotrienes are also released[30] and there is a later increase in histamine during the late nasal reaction[31] More recently, increases in IL-5 and eotaxin have been detected in nasal lavage fluid in the early and late reactions following NAC[21,32].

Filter paper strips that are placed on the turbinates to absorb nasal secretions have proved useful in analysing nasal exudates[33]. The nasal filter paper method has the advantage of directly sampling nasal secretions which are less diluted and can therefore pick up protein signals which are below the detection limits of nasal lavage. The matrix or filter paper method has been used to measure chemokines and cytokines after NAC[17,24,34,35]. However, it should be noted that nasal lavage probably represents an extracellular signal, while nasal sampling by absorption into filter paper strips probably represents both an intracellular and extracellular signal, since cells that adhere to the surface of the filter paper may lyse and release their intracellular contents.

The National Heart and Lung Institute (NHLI) Clinical Studies Unit has been involved in the development of refinements of nasal allergen challenge[36-38]. Subjects with hay

fever and documented skin prick test reactions to Timothy grass pollen are studied when out of season. NAC is performed with Timothy grass pollen (500BU) that is delivered using a validated nasal spray device (Dolphin nasal applicator). At intervals of up to 8hrs after NAC, the nasal exudate is assessed by nasal lavage on the left nostril and nasal filter paper on the right nostril. Lavage is used to measure cell and eosinophil numbers while nasal filter paper eluate is used to measure inflammatory mediators. Responses to single dose and seven days of dosing of nasal steroid have been studied[36-38]. Importantly, it has been noted that there are two phases (early and late) in the responses of IL-4, IL-5 and IL-13 to nasal allergen challenge with Timothy grass pollen. The early phase occurs within the first hour after challenge and the second commences after the first hour. Given that the mechanism of action of OC000459 is thought to be the inhibition of the late phase response to allergen challenge, the effect on the late phase of the IL response will be studied as the primary endpoint.

The Investigator(s) are required to become familiar with the contents of this protocol and of the OC000459 Investigator Brochure version 3.

2. OBJECTIVES

Primary

To assess the effects of OC000459 on levels of IL-4, IL-5 and IL-13 (Th2 cytokines) in nasal filter paper (measured using Luminex) for 1 to 9hr after nasal allergen challenge.

Secondary

To assess the effects of OC000459 on the following parameters after nasal allergen challenge as follows:

- Th2 cytokines at 15 and 30min after nasal allergen challenge.
- Total eosinophil counts (number of eosinophils/ml) in nasal lavage fluid after nasal allergen challenge.
- PGD_2 levels in nasal filter paper after nasal allergen challenge.
- Total nasal symptoms scores after nasal allergen challenge.

To assess the safety of OC000459 in terms of adverse events, vital signs and safety laboratory parameters.

To assess plasma concentrations of OC000459 after five doses of 400mg daily and their relation to inhibition of Th2 cytokines, PGD_2 and eosinophils in nasal fluid after allergen challenge.

3. STUDY DESIGN

3.1. Type of study

The study will be a randomised, double blind, placebo controlled, two way crossover evaluation of the effect of OC000459 on cytokine secretion induced by nasal allergen challenge with Timothy grass extract. Up to 20 (to yield sixteen evaluable) male subjects with a known history of grass pollen related allergic rhinitis and screening positive to allergen challenge with Timothy grass extract by skin prick test and nasal allergen challenge with Timothy grass extract will be included. After five days of dosing with OC000459 400mg or placebo, nasal allergen challenge will be performed

and the following will be measured in the nasal fluids: IL-4, IL-5, IL-13 (primary endpoints, nasal filter paper), PGD_2 (nasal filter paper) and eosinophils (in nasal lavage washings). Nasal symptom scores will also be recorded. There will be a 14-21 day washout period between the first and second treatment periods (Treatment Period 1 and Treatment Period 2) of the study. Safety parameters will be monitored throughout and blood for drug plasma concentrations will be drawn before and after dosing with test article.

3.2 Safety and biological activity endpoints

Biological activity will be assessed as follows:

Primary endpoint:
Levels (AUC and maximum concentration) of IL-4, IL-5 and IL-13 in nasal filter paper (measured using Luminex) after nasal allergen challenge.

Secondary endpoints:
Total eosinophil counts in nasal lavage fluid, PGD_2 concentrations in filter paper after nasal allergen challenge.
Total nasal symptom scores after nasal allergen challenge.
Adverse events, vital signs, concomitant medication and laboratory safety parameters.
Plasma drug concentrations will be related to IL4, 5 and 13, PGD_2 and eosinophil levels in nasal secretions.

3.3. Rationale for study design

This is the first proof of concept study for OC000459 and is undertaken to assess the effects of the compound on the development of inflammatory cytokines in a model that is validated and clinically safe and easy to conduct. A single dose level of 400 mg of OC000459 will be used (the highest dose evaluated in the multiple dose tolerability study in healthy volunteers) and will be compared with placebo. A randomised, balanced crossover is considered to be the most robust design since subjects can act as their own controls and thus reduce variability of comparisons between OC000459 and placebo.

3.4. Study site, duration and recruitment rate

The study site is:

National Heart and Lung Institute Clinical Studies Unit,
First Floor, Royal Brompton Hospital,
Fulham Road,
London SW3 6HP,
United Kingdom.
Telephone: 020 7351 8974
Facsimile: 020 7351 8973

Recruitment is expected to be completed within a three month period and the study is expected to be completed within five months.

3.5 Justification of the proposed dosing level

The dose chosen is based on findings from the study OC000459/003/05 which evaluated doses of up to 400 mg OC000459 administered for up to fourteen days. This dose was well tolerated over a fourteen day period.

4. STUDY POPULATION

4.1 Number of subjects to be studied

Up to 20 subjects will be included to yield 16 who complete the study.

4.2 Entry criteria

4.2.1 Inclusion criteria

1. Males aged 18 to 50 years with a history of symptoms of grass pollen related allergic rhinitis within the previous two years.
2. Subjects must be free from significant cardiac, pulmonary, gastrointestinal, hepatic, renal, haematological, neurological and psychiatric disease as determined by history, physical examination and screening investigations.
3. FEV_1 within normal limits.
4. Atopy defined by a positive cutaneous response (wheal ≥ 3mm compared to control) to Timothy grass pollen.
5. Asymptomatic at screening as characterized by:
   a. Normal appearing nasal mucosa with no active allergic rhinitis.
   b. A total nasal symptom score sheet on study entry so that subjects produce a score of <2 at screening and for 3 days before treatment periods 1 and 2.
6. Non smokers for at least the past 12 months with a pack history ≤ 1 pack years (Pack years = (No of cigarettes smoked/day/20) x No of years smoked).
7. An eosinophilic nasal response after NAC with Timothy grass pollen. Following the NAC and filter paper collection noted above, a nasal lavage will be performed in each nostril at 1hr. Levels of eosinophils must be ≥20,000 eosinophils/ml of nasal lavage fluid in either nostril.
8. Capable of giving informed consent, which includes compliance with the requirements and restrictions listed in the consent form.
9. Available to complete the study.
10. Subjects with a negative urinary drugs of abuse screen, determined within 14 days of the start of the study and throughout the study treatment periods.
11. Negative carbon monoxide test (smokerlyzer) determined within 14 days of treatment period 1, and throughout the study treatment periods.
12. Subjects with a normal 12-lead electrocardiogram (ECG), determined at screening.

4.2.2 Exclusion criteria

1. Medical conditions likely to affect the outcome of the study.
2. Nasal conditions likely to affect the outcome of the study, i.e. nasal septal perforations, nasal polyps, sinus disease, chronic nasal obstruction, or other nasal diseases.
3. Presence of any respiratory disease other than a history of mild stable asthma not requiring treatment and associated with normal lung function (defined as ≥ 90% predicted for height and age).
4. History of immunotherapy in the past 3 years or currently on an immunotherapy treatment course including inhaled or local corticosteroids in the past 28 days.
5. Any infirmity, disability, or geographic location which, in the opinion of the principal investigator, would limit compliance with the protocol.
6. Infection of the upper airways/lower airways, sinus, or ear, including viral infections in the 14 days prior to screening and at the start of each treatment period.

7. Clinically significant abnormality in clinical laboratory tests at screening as determined by the principal investigator.
8. Inability to tolerate lavage correctly with a preliminary nasal lavage at screening.
9. The subject has participated in a study with a new molecular entity during the previous four months or any other trial during the previous three months.
10. The subject regularly, or on average, drinks more than four units of alcohol per day.
11. The subject has a history of testing positive for hepatitis C antibody or hepatitis B surface antigen or for HIV.
12. A history of gastrointestinal disorder likely to influence drug absorption.
13. Receipt of prescribed or over the counter medication within 14 days of the first study day and for the duration of the trial, including vitamins. In particular, all non-steroidal anti-inflammatory drugs (e.g. ibuprofen) are prohibited during this period.
14. Inability to communicate well with the investigator (i.e., language problem, poor mental development or impaired cerebral function).
15. Donation of 450 ml or more blood within the previous 12 weeks.
16. A history of hypersensitivity and/or idiosyncrasy to any of the test compounds or excipients employed in this study.
17. Any previous clinical trial involving the administration of OC000459.
18. Subjects with clinical features suspicious of tuberculosis – weight loss, pyrexia, haemoptysis, purulent sputum, abnormal chest X-ray will be excluded from the study.
19. Unwilling to eat set breakfast.
20. Unwilling to use a reliable form of contraception during the study and for a week after the last exposure to test article.

4.2.3 Withdrawal criteria

1. Subject experiences a serious or intolerable adverse event that prevents him from continuing.
2. Subject incurs a significant protocol violation.
3. Subject requests early discontinuation.
4. Request of the Sponsor.
5. Investigator's request (e.g. if the Investigator considers that the subject's health is compromised by remaining in the study or the subject is not sufficiently co-operative).
6. Subject is lost to follow-up.

5. TREATMENT PLAN AND METHODS

5.1 Study Schedule

Treatment plan and methods

Male healthy volunteers conforming to the selection criteria will be invited to take part in the study. After giving fully informed written consent, they will undergo a complete screening assessment. This screening assessment will not take place more than two weeks prior to the start of dosing or less than seven days.

Subjects will commence dosing with OC000459 400 mg or placebo on day 1 (visit 2 or visit 5) of each treatment period of the study. Blood will be drawn for safety testing (haematology and clinical chemistry) and urine will be obtained for a screen for drugs of abuse. The urine will also be subjected to dipstick analysis for protein, blood,

ketones and urobilinogen. A smokerlyzer test will be performed along with an alcohol breathalyser test. Adverse events and concomitant medications will be noted in the CRF and dosing with the test article will then be done under supervision. Subjects will be given further supplies to take home. Subjects should take each dose (4 capsules in the morning) as soon as they have finished breakfast.

On day 4 of the study period (visit 3 or visit 6), subjects will report to the Clinical Unit at 21.00 hours. Adverse events and concomitant medications will be noted in the CRF for the period since the day 1 visit. A smokerlyzer test will be performed along with an alcohol breathalyser test. A urine sample will also be tested for drugs of abuse. A positive result on any of these three tests (smoking, alcohol, drugs) will make the subject ineligible to continue in the study. On the following morning (day 5), blood will be drawn for safety laboratory testing and plasma drug concentration. A urine sample will be tested by dipstick analysis for protein, blood, ketones and urobilinogen. Vital signs (Supine and standing blood pressure and pulse rate, respiration rate, oral temperature) will be recorded. A high fat breakfast will be served at about 6.30 a.m. The fifth dose of test article will then be taken at 7.00 a.m.

Two hours and thirty minutes after **dosing** a total nasal symptom score will be documented and a nasal lavage will be carried out on both nostrils and discarded. At two hours and forty five minutes after dosing a single filter paper will be taken from the right nostril and nasal lavage will be undertaken in the left nostril. Two hours and fifty five minutes after dosing, blood will be drawn for plasma drug level and blood pressure and heart rate will be recorded. Three hours after **dosing**, a nasal allergen challenge in both nostrils using Timothy grass extract will be carried out.

Following the **nasal allergen challenge**, the following tests will be carried out:

Nasal symptom score at: 30min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, 9hr.

Nasal filter paper (right nostril only) at: 15min, 30min, 1hr, 2hr, 3hr, 4hr, 5hr, 6hr, 7hr, 8hr, 9hr.

Nasal lavage (left nostril only) at: 30min, 1hr, 2hr, 4hr, 6hr, 8hr.

Blood will be drawn for measurement of plasma concentrations of test article at: 2hr, 4hr, 6hr, 8hr.

Heart rate and blood pressure will be recorded at the following times: 2hr, 4hr, 6hr, 8hr.

Adverse events and concomitant medications will be recorded throughout the study period.

Finally, sampling for laboratory safety tests (urine dipstick analysis for protein, blood, ketones and urobilinogen and blood for haematology and clinical chemistry) will be carried out at least 12 hours after dosing.

Following completion of all study related procedures that day, subjects may go home unless they have suffered an adverse event that requires further hospital treatment.

They will be contacted by a member of staff from the Clinical Studies Unit the following morning (Visit 4 and Visit 7) to document any new adverse events or changes in concomitant medications.

The second treatment period (Treatment Period 2) of the study will commence 14-21 days after the final blood samples in the first treatment period (Treatment Period 1) have been drawn and will be identical to the first treatment period (Treatment Period 1) of the study.

A final follow-up visit will be conducted four weeks after the last dose of test article in Treatment Period 2.

Total blood loss for this study is expected to be approximately 120 ml.

5.2 Allocation of treatments and randomisation procedures

Randomisation codes will be generated and will be lodged with the pharmacy department of the Royal Brompton Hospital as well as with the Clinical Studies Unit (see section 8.5 for a full explanation).

The randomisation will be blocked to provide a balanced allocation of subjects to the two sequence groups:

A. OC000459 in the first study treatment period (Treatment Period 1) and placebo in the second study treatment period (Treatment Period 2)
B. Placebo in the first study treatment period (Treatment Period 1) and OC000459 in the second study treatment period (Treatment Period 2).

Of the 20 subjects in the study, 10 will be in sequence group A and 10 will be in sequence group B.

Subjects successfully completing the screening assessments and selection procedures will be randomised into the study and will receive the study medication supplies corresponding to the next free number on the randomisation schedule.

5.3 Study Medication Administration

Study medication (test article) will be packaged for dosing over five days as a single daily dose taken in the morning with water. Subjects will be instructed to take their study medications (4 capsules daily) straight after taking breakfast.

OC000459 and matching placebo will be presented as gelatine capsules (size 0) 100 mg strength.

The fifth dose will always be taken 15 minutes after breakfast under supervision at the Clinical Studies Unit on day 5 of each treatment period. This will be a standardised full fat breakfast.

5.4 Restrictions

5.4.1 Prior and Concomitant Therapy

Prescribed medications are not allowed for two weeks prior to the subject's inclusion in the trial or for the duration of the subject's participation in the trial. Over the counter medications including vitamins should also not be taken for two weeks prior to the subject's inclusion in the trial or for the duration of the subject's participation in the trial. If a subject has taken over the counter medications during the two weeks before the study start, the reason for taking them will be noted in the Case Report Form. Inclusion of subjects who have taken over the counter medications during this period will be reviewed on a case-by-case basis in relation to the safety aspects and

objectives of this study. **Non-steroidal anti-inflammatory drugs are not allowed for two weeks prior to and during the course of the trial.**

Over the counter medications should be avoided throughout the duration of the study dosing periods, with the exception of paracetamol, which may be taken as an analgesic to a maximum of 2g in 24hr with the agreement of the Investigator. If intake of a medication should become necessary for any reason during the course of the trial, the subjects must inform the investigator immediately who will note the drug, the dose and the time of intake in the CRF.

5.4.2 Other Restrictions

Caffeine, alcohol and exercise are not permitted during days 1-5 of each treatment period of the study. Drugs of abuse and smoking are not permitted at any time prior to or until the end of Treatment Period 2.

5.5 Specific procedures

5.5.1 Screening and post-study procedures

5.5.1.1. Screening and selection procedures

Male healthy subjects conforming to the selection criteria will be invited to take part in the study. After giving fully informed written consent, they will undergo a complete screening assessment to include: full medical history (including a history of alcohol and cigarette use), physical examination, (which will include the cardiovascular, respiratory, haemopoietic, central and peripheral nervous, gastrointestinal, endocrine and renal systems and FEV_1), height, weight, supine and standing blood pressure and pulse rate, respiration rate, oral temperature (vital signs), 12-lead ECG and laboratory safety tests (clinical chemistry, haematology, dipstick urinalysis for protein, blood, ketones and urobilinogen) and urine will also be tested for drugs of abuse (drug screen). A smokerlyzer test will be undertaken and must be negative. A skin prick test for sensitivity to Timothy grass will be undertaken. In addition, a nasal allergen challenge with Timothy grass pollen will be conducted and nasal lavage will be performed in both nostrils one hour post challenge. Any concomitant medications will also be recorded. This screen will not take place more than two weeks prior to the start of dosing or less than seven days.

5.5.1.2. Post-study procedures

Four weeks after completion of dosing subjects will be asked to return for a post-study follow up visit. At this time, a physical examination will be undertaken, supine and standing blood pressure and pulse rate, respiration rate and oral temperature (vital signs), a 12-lead ECG and laboratory safety tests (clinical chemistry, haematology, dipstick urinalysis for protein, blood, ketones and urobilinogen). Any adverse events and concomitant medications since their previous visit will be followed up and recorded.

5.5.2 Assessments for biological activity

5.5.2.1 Nasal allergen challenge

Skin/Epidermal Prick Test

Subjects have a positive skin prick test reaction, with a raised wheal of $\geq$3mm in diameter when compared to saline negative control and to histamine positive control.

Soluprick SQ (ALK 225) Timothy grass pollen, Phleum pratense, ALK Abello freeze dried extract, cat. no. 1001487.

Nasal Examination

Prior to nasal allergen challenge (NAC) or any nasal procedure, a full and careful assessment of nasal anatomy is performed by a fully trained member of the medical staff. Using an illuminated head mirror or head lamp and a Thudichum's speculum the nasal cavity is inspected for anatomical variations, septal defects and nasal polyps. The nasal mucosa would also be assessed for signs of inflammation.

Nasal Allergen Challenge (NAC)

Nasal allergen challenge is performed with Timothy grass pollen

Aquagen SQ (ALK 225) Timothy grass pollen, Phleum pratense, ALK Abello freeze dried extract, Cat. no. 1001862.

Application of the allergen to the nasal mucosa is undertaken using a nasal pump spray (Bidose or Dolphin nasal applicator, Valois).

A total dose of 1000 biological units is given as 500BU to each nostril. A dose of 320BU has previously been shown to provoke both immediate and late-phase responses [39]

Nasal lavage for cell counts and cytology

A. Nasal lavage procedure

Nasal filter paper adsorption of secretions (right nostril) is performed prior to nasal lavage (left nostril).

Lavage Materials

1. Syringe (10ml) and needle
2. Dulbecco's Phosphate Buffered Saline (PBS), without magnesium chloride and calcium chloride (Sigma, Product No. D-8537)
3. Bowl and ice packs for sample storage during collection
4. 50 ml polypropylene centrifuge tube (Falcon tube)
5. Tissues & towel
6. Bowl for waste
7. Nasal adapter or olive (Mefar, Tel: 01379 741 525 small size product order no. 479.003, medium size product order no. 479.004, large size product order no. 479.005). Extra large olive adaptors have been manufactured by the Clinical Engineering Department of the Royal Brompton Hospital (Mr Keith Wilson Tel 020 7351 8662)

Lavage Procedure

1. Nasal lavage with Dulbecco's Phosphate Buffered Saline (PBS) (Sigma, Product No. D-8537) will be performed using a 10ml syringe attached by tubing to a nasal adapter or olive.
2. The nasal olive of an appropriate size attached to a pre-filled syringe is given to the subject who is requested to place it gently but firmly into their nasal opening so as to make a close fitting airtight seal and later prevent leak of lavage fluid.
3. The subject is seated in a forward-flexed neck position (60° from the upright position) to prevent fluid from reaching the nasopharynx.

Preliminary discarded lavage

To ensure comparable baseline nasal washings and filter paper sampling, sterile PBS lavage fluid (5ml) is introduced into the nasal cavity, and the fluid

is withdrawn into the syringe and flushed back into the nasal cavity 20 times over a 1 min period. The nasal lavage fluid recovered is discarded only for this preliminary wash. Following this wash there is at least a 5 min wait before baseline sampling.

Subsequent nasal lavages

PBS lavage fluid (5ml) is introduced into the nasal cavity, and the fluid is withdrawn into the syringe and flushed back into the nasal cavity 20 times over a 1 minute period. The nasal lavage sample is collected in a 15ml polypropylene centrifuge tube and placed immediately on ice. The total volume of lavage is recorded.

Laboratory Personnel Safety

1. Nasal lavage processing must only be performed by a laboratory scientist experienced in lavage processing methodology. Nasal secretions may contain Mycobacterium tuberculosis and other class III pathogens.
2. Subjects with clinical features suspicious of tuberculosis – weight loss, pyrexia, haemoptysis, purulent sputum, abnormal chest X-ray will be excluded from nasal lavage.
3. Sealed buckets/rotors should be used for centrifugation (including Cytospin) steps.
4. Appropriate disinfection procedures should be carried out for all non-disposable equipment (Hycolin) and work surfaces (Virkon).

Processing Materials

1. Dithiothreitol (DTT) (Sigma, Product No. D-9779) Store dry powder at 2-8 °C.
2. 1% DTT Stock solution: 0.1g in 10 ml distilled water. Keep dark, refrigerated for up to 30 days.
3. 0.22µm pore size cellulose acetate filters (Costar spin-X ®) Corning Incorporated Cat No. MPA-150-020W
4. Dulbecco's Phosphate Buffered Saline (PBS), without magnesium chloride and calcium chloride (Sigma, Product No. D-8537)
5. Trypan Blue, 0.4% (Sigma, Product No. T-8154)
6. Ethanol, 100% (BDH, Product No. 28304)
7. D.P.X. Mounting Medium RI 1.5 (Merck Cat No. 36029)
8. Diff-Quik® staining kit
   (Manufacturer: Dade Behring. UK Distributor: Gamidor Ltd, 66 Milton Park, Abingdon, Oxon, OX14 4RX. Tel: (01235) 832266.Cat No. 130832)
9. Polypropylene centrifuge tubes, 15 ml and 50 ml
10. Disposable 3 ml plastic pipettes
11. 1.5 ml cryotubes
12. Shandon Cytospin Filter cards (Shandon, Cat No. 59910022)
13. Cytoslide™ coated microscope slides (Shandon, Cat No. 5991056)
14. Cover slips (BDH), size 0 (18x18 mm – Cat no. 406/0187/21)
15. Cover slips (BDH), size 1 (24x50 mm – Cat no. 406/0188/82)

Processing Equipment

1. Pipettes, 5-50 µl, 50-250 µl, 200-1000 µl, 1-5 ml (Jencons Sealpette)
2. Vortex mixer (Vorsicht)
3. Rolling mixer (Denly Spiramix 5)
4. Refrigerated centrifuge (Sorvall RT6000D)
5. Improved Neubauer haemocytometer (BDH)
6. Light microscope, X10 / X40 magnification (Leitz Wetzlar)
7. Shandon Cytospin® 3 Cell Preparation System

8. Shandon Cytospin sample holders: steel slide clips Cat No. 59910052
9. Funnel chambers Cat No. 59910021
10. Microscope slide transport box (Merck Cat No. 406/0197/00)

Merck/BDH Sales: 0800 223344 Sigma Sales: 0800 717181 Shandon Sales: 0800 0189396)

Prompt processing: Nasal lavage should be processed immediately: in unusual circumstances lavage fluid can be kept on ice for a maximum of 2 hours.

Nasal lavage collection: The nasal lavage sample is collected in a 15 ml polypropylene centrifuge tube and placed immediately on ice. The total volume of lavage recovered is retained and the volume recorded.

Centrifugation: The total volume of nasal lavage recovered is centrifuged at 4°C for 10 minutes at 400*g* (1500rpm).

Lavage Supernatant aliquots

The supernatant is then aliquotted using 3ml disposable wide bore pipette into another 15 ml polypropylene centrifuge tube and this is mixed using a vortex to ensure that the sample is homogeneous. 500µL aliquots are transferred to labelled cryotubes. The cryotubes are labelled using a cryopen with:

Subject Initials
Subject Identification Number
Specimen Identification Number
Centre number
Visit number
Sampling time-point

These are stored at –80°C for later analysis. The lavage supernatants are centrifuged through a 0.22µm pore size cellulose acetate filters (Costar spin-X ®) at 4°C for 30 min at 3200rpm to remove residual mucus before analysis.

Lavage Cell Counts and Cytospin

Resuspension: The remaining cell pellet is resuspended in 5mls of 0.1% DTT.

Reagents: A 1% stock Dithiothreitol (DTT) is made up monthly and kept in the fridge (e.g. 0.1g of DTT in 10ml of PBS). On the day of processing this 1% stock is diluted 1 in 10 (e.g. 1ml of the 1% DTT stock is added to 9ml of PBS).

Liquefaction: This cell suspension is gently aspirated with a 3 ml disposable wide bore plastic pipette. The sample is gently agitated on a rolling mixer for 10 minutes. It may be necessary to aspirate further to ensure homogeneity.

Centrifugation: The nasal lavage is again centrifuged at 4°C for 10 minutes at 400*g* (1500rpm). The DTT containing supernatant is removed using a 3ml disposable wide bore pipette and discarded.

Resuspension: The remaining pellet is resuspended in PBS to make a total volume of 110µl.

Haemocytometry: 10µl of mucus cell suspension is added to 10µl of trypan blue (1 in 2 dilution). 10µl of the mixture is placed under the microscope for cell counting using an improved Neubauer haemocytometer. A size number 1 cover slip is used. If the

cells are too dense for an accurate count, the procedure is repeated with additional dilution until satisfactory for an accurate count. The dilution is noted. Cells are counted in the bottom left, middle and top right quadrants of the haemocytometer grid.

Calculation: The total leukocyte count per ml is calculated, taking into account the dilution factor of trypan blue, by using the following formula:

Total leukocyte count (cells/ml) = (Average no of viable + dead leukocyte) X trypan blue dilution factor X 2000.

Between each use the haemocytometer is stored in methanol.

Cytospin preparation: After calculation of total cell count, the lavage cell suspension is adjusted to 200,000 leukocytes/ml. One cytospin block is prepared, using labelled coated slides. 100µl aliquots are loaded onto the blocks. If the dilution factor calculated is less than or equal to two then the sample should not be diluted and all of the residual 200µl cell suspension should be loaded onto a single block and one slide should be produced. The cytospin slides are produced by spinning at 450 rpm, medium acceleration, for 3 minutes. Each slide must be labelled as follows:
Subject Initials
Subject Identification Number
Specimen Identification Number
Centre number
Visit number
Sampling time-point

Staining: The four slides are left to air-dry for 30 min, and then fixed in methanol. One slide is stored at room temperature. The remaining slides are stained with Diff-Quik stain, mounted using size 0 cover slip and DPX, and stored at room temperature.

Expression of lavage results: Differential cell counts are determined by assessment of 400 leukocytes on the cytospin. This is converted to a percentage of eosinophils and the total number of eosinophils calculated.

B. Nasal Filter Paper for Assay of Inflammatory Mediators

Materials

1. Filter paper (Whatman No. 42; Whatman Paper Ltd, Maidstone, UK)
2. Dulbecco's phosphate buffered saline (PBS), without magnesium chloride and calcium chloride (Sigma, Product No. D-8537)
3. Bowl and ice packs for sample storage during collection
4. 50 ml polypropylene centrifuge tube (Falcon tube)
5. Tissues and towel
6. Bowl for waste

Filter Paper Procedure

Preliminary discarded nasal wash (see above) to remove mucus.

a. Two filter strips are cut with scissors to size 7 x 30mm/strip (Whatman No. 42; Whatman Paper Ltd, Maidstone, UK). The sharp corners are trimmed. Pairs of strips are **weighed** before adsorption of nasal secretions.

b. An Eppendorf containing Luminex assay buffer (500µl) is **weighed**.

c. With the Subject sitting comfortably upright, and the head extended backwards, the filter papers are placed together back-to-back on the lateral wall of the RIGHT nasal cavity using blunt-ended forceps. Strips are left to absorb nasal secretions for 2 min.

d. The 2 strips are removed together with blunt-ended forceps, folded in half and placed into the pre-weighed Eppendorf containing Luminex assay buffer (500µL). The tube is **reweighed** after the addition of the filter papers.

e. The papers are completely submerged in assay buffer and then mixed using a vortex for 15 seconds and then the rolling mixer for 10 minutes to allow mediators to elute.

f. After centrifugation at 4°C for 10 minutes at 400*g* (1500rpm), the eluate is collected in fractions of 100µl and stored at -80°C.

g. Luminex analysis. After thawing, a BSA solution (20%) to give a final 2% solution is added to the eluate.

h. The mixture is then centrifuged through a 0.22µm pore size cellulose acetate filters (Costar spin-X ®) at 4°C for 30 minutes at 3200rpm to remove residual mucus.

i. The mixture is then assessed on the Luminex 100 IS® analyser. Results are generally expressed in terms of pg/ml or pM, relating usually to the 500µl of assay buffer eluate. Results can be expressed in terms of the volume of adsorbed secretion.

Filter Paper Processing

1. After removing and reweighing, the two strips are placed together into an assay buffer (500µl of PBS pH 7.4, 1%BSA, 0.05% Tween® -20, 0.05% sodium azide).
2. The papers are mixed using a vortex for 15 seconds and then the rolling mixer for 10 minutes to allow mediators to elute.
3. After centrifugation at 4°C for 10 minutes at 400*g* (1500rpm), the eluate is collected and stored at -70°C for later analysis using Luminex 100 IS® analyser.
4. The eluate is centrifuged through a 0.22µm pore size cellulose acetate filters (Costar spin-X ®) at 4°C for 30 min at 3200rpm to remove residual mucus before analysis
5. Results are generally expressed in terms of pg/ml or pM, relating usually to the 500µl of assay buffer eluate. Results can be expressed in terms of the volume of adsorbed secretion.

C. Total nasal symptom scores

Nasal symptoms will be scored in terms of nasal obstruction, rhinorrhea, nasal itch and sneezing. All symptoms are scored on a four point scale and the total score for each time point will be assessed. An example score sheet is presented in Appendix 2.

5.5.3 Assessments for safety

5.5.3.1 Clinical

Heart rate, lying and standing systolic and diastolic blood pressure, body temperature and respiratory rate will be measured in singlicate (once) using well calibrated electronic machines for all measures except respiratory rate. Spontaneously reported adverse events and concomitant medications will be recorded throughout the study. Twelve lead ECGs will be obtained at the screening visit, at day 1 of Treatment Period 2 (Visit 5) and at the final follow up visit.

5.5.3.2 Laboratory

A urine screen for drugs of abuse, an alcohol breathalyser test and a smokerlyzer test will also be obtained at screening and at each subsequent attendance in the unit during the treatment periods.

Laboratory safety assessments at screening, on days 1 and 5 (a.m. and evening) of each treatment period of the study and at follow up will consist of haematology measurements (haemoglobin, haematocrit, MCV, MCHC, total and differential white cell count, platelet count) and clinical chemistry measurements (urea, sodium, potassium, chloride, bicarbonate, creatinine, total bilirubin, AST, ALT, ALP, serum proteins, uric acid, calcium, phosphate, glucose).

Urine dipstick measurements will be made for protein, blood, ketones and urobilinogen and will be performed on the Clinical Studies Unit.

5.5.4. Drug in plasma blood sampling

All blood samples must be drawn within ± 5 minutes of the time stipulated in the protocol. The times of blood sampling, centrifugation and placing in the freezer should be recorded in a laboratory notebook. The analytical laboratory for the pharmacokinetic blood samples will be BioDynamics, Rushden, Northants, NN10 6ER.

Blood samples (2x1 ml) will be taken into 2 ml plastic (Vacutainer) tubes containing lithium heparin and pre-cooled on water/ice (+4ºC). Blood samples will be kept on water/ice and will be centrifuged at about 2500xg for 10 minutes at 4ºC, within 15-30 minutes of collection (15 minutes is preferred). The resulting plasma will be transferred to two polypropylene vials and immediately frozen. The vials will be kept frozen at -20ºC until transferred to BioDynamics for assay. Only one of these aliquots will be shipped to BioDynamics initially for determination of OC000459 by a validated LC-MS/MS assay. The other must be retained on site, stored at -20°C until required. Each sample tube will be clearly labelled with indelible ink with the following information: Plasma. Study number (OC000459/005/05), subject number, time point and dose period and a unique nine-digit code. The actual sampling time after dosing will also be provided at the time of shipment.

Once the collection is complete, samples will be transported deep-frozen (-80°C) and packed in sufficient dry ice to ensure samples arrive frozen at BioDynamics. The shipment documentation should identify whether this is the first or the second set of samples. The outside of the package must clearly indicate that the samples must be placed in a -20ºC deep freezer immediately on arrival at the destination.

The packages should be addressed to:
Mr Mike Redrup
BioDynamics,
Pegasus Way,

Crown Business Park,
Rushden,
Northants NN10 6ER, UK.
Telephone: 01933 319900
Facsimile: 01933 319990
Email: Mike.Redrup@BioDynamics.co.uk

Prior to shipment, Mr Mike Redrup will be informed of the intended shipment date and date of arrival of the samples at BioDynamics by email or facsimile.

5.6 Compliance checks

Source data will be verified with 100% checks being run on compliance with entry criteria, informed consent documents, CRF signatures, evaluability status, adverse events, withdrawals, out of range laboratory values, 12-lead ECGs, safety blood parameters, biological activity variables and compliance with the medication. A 30% check will be conducted for vital signs, test article blood sampling times and concomitant medications.

In order to be evaluable, volunteers must have given written informed consent, complied with all the entry criteria and the dosing regimen and have completed 90% of all protocol driven measurements. The subjects may be replaced.

Non compliance and the reasons for it are to be documented in the CRF.

5.7 Stopping rules

The trial may be stopped if serious and unexpected adverse events occur which are believed to be OC000459 related. Individual subjects may also be withdrawn from the trial in the event of unacceptable toxicity or non-compliance such that it would affect the interpretation of the data (see section 4.2.3).

6. STUDY MATERIALS

6.1 Study medications

The Investigator and the Royal Brompton Hospital Pharmacy are responsible for investigational product accountability. To this end, all clinical trial supplies will be delivered to and be the responsibility of a suitably qualified and authorised pharmacist, who will document drug disposition and accountability for the duration of the trial. The pharmacist will also perform tablet counts on returned study medications to assess compliance.

6.2 Packaging and labelling

The study medication will be packaged and labelled for each subject by Penn Pharmaceutical Services [Tredegar, Gwent, NP22 3AA Tel. 01495 711222] according to Annex 13, Rev 1 (manufacture of investigational medicinal products) of the EC guide to Good Manufacturing Practice. Supplies will be packed in such a way that the subjects, investigator/study staff, sponsor staff and their representatives are blind to their contents.

6.3 Storage and disposition of study medications

Clinical trial supplies must be stored at 15-25ºC in the pharmacy. They must 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.

At the end of the trial, the clinical trial monitor will review the drug-dispensing log and reconcile it with the remaining stock of study medication. Eight weeks after completion of the study, any left over study medication will be returned to Penn Pharmaceutical Services for destruction.

6.4 Precautions/overdose

OC000459 is an investigational agent and is contraindicated for all conditions other than those mentioned in this protocol and the Investigator Brochure.

Should an overdose occur, there is no known antidote. Symptoms and signs attributed to the overdose should be treated symptomatically.

OC000459 has been shown in preclinical studies to cause small elevations in fed blood glucose and prolongation of the QRS complex in a single animal. These abnormalities have not been observed in studies OC000459/001/04 and OC000459/003/05 or in longer term 28 day rat and dog toxicology studies. The clinical significance of these findings is unclear.

6.5 Other study supplies.

Case record forms (CRFs) will be supplied by Akos Limited. Each page of the CRF will be supplied in duplicate NCR sheets. The investigator must keep all CRF supplies, both completed and blank, in a secure place.

7. ADVERSE EVENTS

7.1 Definitions

An adverse event is any untoward medical occurrence in a subject administered a pharmaceutical product that does not necessarily have to have a causal relationship with the treatment. All adverse events must be described in the appropriate section of the CRF and their severity and putative relationship to the study medication noted. Definitions of severity are as follows:

Mild: does not interfere with the conduct of the study, resolves spontaneously, does not need medication or any other therapy.

Moderate: requires treatment, interferes temporarily with the conduct of the study.

Severe: forces withdrawal from the study

Serious: death, life threatening, requires or prolongs hospitalisation, results in persistent or significant disability/incapacity, overdose, or is a congenital anomaly/birth defect

Definitions of relationship to study medication are as follows:

Unrelated: bears no relation to timing of medication and similar to symptoms or signs expected in the disease process and does not recur on rechallenge.

Possibly: bears relation to timing of medication and similar to symptoms or signs expected in the disease process and does not recur on rechallenge.

Probably: bears clear relation to timing of medication and distinct from symptoms or signs expected in the disease process and does not recur on rechallenge

Definitely: bears clear relation to timing of medication and distinct from symptoms or signs expected in the disease process and recurs on rechallenge.

Adverse events may also be expected or unexpected. If expected, they will be mentioned in the Investigator Brochure

7.2 Serious and unexpected adverse events

All serious adverse events must be reported promptly to the Sponsor and in any case not later than 24 hours after their occurrence. The report must be made by telephone or fax to the following contact at the following numbers:

Ms Maria Hilling
Akos Limited
Telephone: 01582 716312
Facsimile: 01582 716327

The Investigator must also complete a serious adverse event form and transfer it to Akos Limited as soon as is possible.

Akos Limited will report all serious, related and unexpected adverse events to the MHRA within 7 days of being notified by the investigator.

7.3 Withdrawals due to adverse events.

Any study subject may be withdrawn from the study at any time at the discretion of the investigator or the request of the subject (see section 4.2.3). The reason for doing so must be clearly documented in the CRF and, if the withdrawal is due to an adverse event, the pertinent page of the CRF must be completed. The Investigator should use his best efforts to follow up the subject following withdrawal from the trial and the appropriate follow up portion of the CRF should be completed at that time.

In general, subjects withdrawn early from the study will be replaced. If withdrawal is caused by an adverse event, which the Investigator considers may be related to the study compound, the subject will not be replaced without reference to the ethics committee responsible for the study.

In the event of any abnormalities considered to be clinically significant by the investigating physician, subjects will be followed up with appropriate medical management until values are considered to be clinically acceptable. Referral or collaborative care will be organised if considered necessary.

8. DATA MANAGEMENT AND STATISTICAL ANALYSES

8.1 Rationale for sample size

The sample size for this protocol was estimated using unpublished data provided by the Chief Investigator from a within-subject reproducibility nasal allergen challenge study, with no drug intervention, on 8 subjects. For these subjects the within-subject variability was greater for levels of IL-4 in nasal filter paper, than for levels of IL-5 and IL-13. The area under the response curve for IL-4 from 1 to 8 hours post nasal allergen challenge (AUC(1-8)) had a standard deviation of within-subject differences

(SD) which was 13.4% of the mean AUC(1-8). These data also suggest a similar relationship between mean response and SD for the maximum IL-4 level from 1 to 8 hours post nasal allergen challenge (Max(1-8)).

In view of the small size of the unpublished data set and the need to incorporate additional within-subject variability introduced by the effects of OC000459, it was felt prudent to double the estimate of SD in the sample sizing for this protocol. With this assumption and using a 5% significance level, 16 subjects is the requirement for a crossover study to achieve >90% power of detecting a 25% reduction in AUC.

In this protocol, the period of observation is extended to 9 hours post nasal allergen challenge, rather than the 8 hours used in the unpublished data used for sample sizing. It is anticipated that the relationship between response and within-subject variability for AUC and Max will not be substantially affected by this change.

8.2 Statistical analysis

The primary objective of the study is to assess the effects of OC000459 on levels of IL-4, IL-5 and IL-13 (Th2 cytokines) in nasal filter paper after nasal allergen challenge (NAC). The primary analyses of these effects will be based on the area under the curve of these levels from 1 to 9 hours after NAC (AUC(1-9)) and the maximum level from 1 to 9 hours after NAC (Max(1-9)). These measures of response will be analysed using a two-way crossover analysis with treatment and period as factors and investigating OC000459 versus placebo differences. In addition the IL-4, IL-5 and IL-13 response profiles for OC000459 and placebo will be summarised and compared at each time-point pre-NAC and over the 9 hour period after NAC.

Similar approaches will be used for the analysis and summary of the secondary measures, including Th2 cytokines at 15 and 30 min after NAC, eosinophil counts in naval lavage fluid, PGD2 levels in nasal filter paper, and total nasal symptoms scores.

The relationship between plasma concentrations of OC000459 and the above measures of effect will also be summarised.

In addition, further exploratory analyses will be carried out to fully assess the effects of OC000459.

Subject disposition, demographic and baseline data will be summarised by sequence group and overall. Adverse events, concomitant medication, vital signs and laboratory safety findings will be summarised by treatment group. All safety data will be listed.

Further detail of the statistical analyses and data presentations to be used in reporting the study will be provided in a Statistical Analysis Plan which will be finalised prior to breaking the blind.

8.3 Criteria for eligibility of subject data

In order to be evaluable, subjects must have given written informed consent, complied with all the entry criteria and the dosing regimen and have completed 90% of all protocol driven measurements. Those who do not will be considered to be major protocol violations in the biological activity analyses.

All subjects, including those who drop out for any reason and those not compliant with the protocol and who receive OC000459/placebo will be evaluated for safety. Subjects not evaluable for safety for whatever reason will be identified in the safety data listings.

The safety population will consist of all subjects who received at least one dose of study medication.

8.4 Case report forms (CRFs)

Data must be entered onto the case report forms completely, legibly and in timely fashion by the investigator his/her designees. The Investigator will also verify that all the data contained on these forms are accurate and will sign the forms once they are completed. Where source data are available, these will be checked against the data in the CRF.

CRFs always remain the property of the Sponsor and must be available for review and retrieval by Sponsor staff at any time. Investigators are requested to enter data promptly onto CRFs in order to facilitate review, monitoring and correction of CRFs in a timely fashion.

Any subsequent alterations to the data must be made by striking out the previous entry with a single line and by writing the new value next to it. All such changes must be initialled and dated by the individual completing the CRF. Whiting or scribbling out of errors is not acceptable.

When the monitor reviews the CRFs, certain queries may arise. These will be documented in writing and the resolution will also be noted in writing. Copies of these query resolution forms will be kept in both the Investigator's and the Sponsor's archive.

Once CRFs have been completed, the top copy of each CRF page will be collected for data entry and analysis at Akos Limited. The bottom copy of the CRF page will be retained at the investigator site. At the end of the study all original CRF pages will be returned to the Sponsor for archiving. The Investigator will retain all CRFs and other study related documents in a secure but accessible place.

8.5 Double blind codes

A randomised code list will be prepared by an independent consultant statistician (Trevor Lewis, TLwise Consulting Ltd, Tel 07766 023710) using an industry standard computer package and will be provided to Penn Pharmaceutical Services. As well as providing the already packaged and labelled clinical study medication to the pharmacy at the Royal Brompton Hospital, Penn Pharmaceutical Services will also supply the Clinical Studies Unit with individual envelopes for each subject containing the sequence code which identifies the order of study medication in the two Treatment Periods [Group A (OC000459 followed by placebo) or Group B (placebo followed by OC000459)] before the trial starts. These must be held in a secure place within the CSU. The double blind code must not be broken except in the case of severe and serious adverse events where knowledge of the subject's study medication would be of benefit to therapy. The Medical Monitor must be contacted immediately by the investigator for a discussion of the circumstances if a code break is deemed necessary. If a code is broken, the time, date and reason and the name of the person breaking the code must be recorded in the CRF and on the envelope.

8.6 Criteria for early termination of the trial

The trial may be terminated early if suspected unexpected serious adverse reactions (SUSARs) occur in any of the healthy subjects (see section 5.7). If this occurs, the Principal Investigator will discuss next steps with the Sponsor and the Medical Monitor.

9. ETHICAL CONSIDERATIONS

9.1 Subject information sheets and consent forms

All subjects invited to participate in a clinical trial are entitled to make their decision based on the fullest amount of information available at the time. In order to make that choice, they will be given a written document in English expressed in clear concise lay language to consider. The document will previously have been approved by the Main REC and may be updated as new important information becomes available that would affect a subject's willingness to participate or continue in the trial.

This document will tell potential subjects about the nature of the indication and the drug, its efficacy and safety profile in animals and man, the human experience to date and the route of administration. It will also outline the numbers of subjects in the trial and the steps of the protocol as they will apply to the individual including number of visits, number of procedures including venepunctures and types of measurements to be performed so that the individual has a clear picture of the risks, inconveniences and benefits that may accrue from the trial. If subjects are to be reimbursed expenses, the sums involved must be made known.

The individual must be made aware that he may refuse to join the trial or may withdraw at any time without prejudicing further medical care and that he is covered by the Sponsor's indemnity insurance in the event of a trial related injury. A contact with whom suspected trial related injuries may be discussed must be given. Individuals must also know that their personal medical records may be reviewed in confidence by the Sponsor's staff or representatives and by Regulatory Authorities and Ethics Committees from time to time and that personal information about them will be held on a confidential database. Conditions for ensuring the anonymity of the data and the security and confidentiality of the database should be explained.

Consent will always be given in writing after the subject has had adequate time to reflect on the information and to ask further questions if need be. The subject will sign and date the consent form.

9.2 Main Research Ethics Committee review.

Main RECs will conform to the standards of ICH E6. They will be provided with the appropriate COREC form, copies of the protocol, any amendments, subject information and consent forms, subject recruitment procedures, details of payments to subjects (where relevant), the Investigator Brochure, the Investigator's Curriculum Vitae and Clinical Trial Indemnity Insurance Documents (if requested) for review. Approval will always be given in writing and within a reasonable timeframe. The approval documents should clearly identify the protocol by title and Sponsor's number, identify the committee members present when the approval was granted and list any required amendments with the reasons for them. If a protocol is refused approval, the reasons will be given in writing. Refusals will be notified to the MHRA.

No trial may begin and clinical trial supplies will not be shipped until written approval is received by the Sponsor. The Main REC will also provide a list of its members, their affiliations and its written procedures if requested to the Sponsor in confidence

as part of the pre study documentation. The Main REC must keep records of all its procedures and decisions for at least three years after completion of the trial and make them available on request to representatives of regulatory authorities.

10. REGULATORY REQUIREMENTS AND SPONSOR / INVESTIGATOR OBLIGATIONS

This study will be conducted in accordance with the Good Clinical Practice (GCP) Guidelines as issued by the International Conference on Harmonisation (ICH E6, 1996), the Declaration of Helsinki (1996) and The U.K. Statutory Instrument which incorporates the European Clinical Trial Directive (Directive 2001/20/EC). To ensure compliance with the guidelines, the study will be audited by third parties including independent auditors and possibly Regulatory Authorities. The Investigator agrees, by written consent to this protocol, to co-operate fully with compliance checks by allowing access to all documentation by authorised individuals.

The study will not begin until written approval is received from the MHRA and the Main REC.

10.1 Study initiation

It is essential that all personnel concerned with the trial understand their duties and responsibilities fully. In order to facilitate this process, the Sponsor will conduct site initiation visits prior to first subject recruitment and after Main REC approval has been obtained. It is expected that all personnel involved will attend this meeting and will familiarise themselves with the protocol, the case report form and the principles of GCP which will be implemented during the trial.

10.2 Monitoring

The purposes of clinical trial monitoring are to verify that the rights and well-being of human subjects are protected, that reported trial data are accurate, complete and verifiable from source documents and that conduct of the study is in accordance with current GCP and regulatory requirements. In order to assist with the collection in a timely fashion of accurate, verified data which are in accordance with the protocol, monitors will visit the study site regularly. They will review source documents and compare them with data contained in the CRF. If inconsistencies occur, these queries will be answered by the Investigator. The monitors will also check subject accrual, drug dispensing procedures and logs, lists of persons to whom clinical trial related activities have been delegated, relevant communications with family physicians, freezers and other equipment as necessary. They will also visit associated laboratories to ensure their continuing compliance with the protocol and with GLP and deal with any problems arising in the course of the study. The Investigator and others involved in the study must make adequate time available to be present at these visits.

The study monitor will be:

Akos Limited,
The Coach House,
The Grove,
Piper's Lane,
Harpenden
Hertfordshire AL5 1AH
Telephone: 01582 766339
Facsimile: 01582 764327

10.3 Documentation and record keeping

Essential records must be retained by the trial site for at least two years after the last approval in an ICH region and until there are no pending or contemplated marketing applications in an ICH region OR at least two years have elapsed since the formal discontinuation of clinical development of the investigational product. No records may be destroyed or moved without the Sponsor's written permission. The Sponsor will archive and retain all documents pertaining to the study for the lifetime of the product under investigation and final study reports will be kept for a further five years.

10.4 Clinical study report

This clinical study will be summarised by Akos Limited, reviewed and approved by the Sponsor/Medical Monitor and a written, final audited report must be retained on file. This report will include discussions of the study objectives, methodology, findings and conclusions. The Principal Investigator will be asked to review and comment on the draft report and will be required to sign the final version. This report must be archived with all other study-related documents.

10.5 Termination of the study

At the end of a clinical study, the Investigator must return to the Sponsor all outstanding CRFs, unused clinical trial supplies and loaned equipment unless other arrangements have been made.

A clinical study may be terminated prematurely by the Principal Investigator by giving 30 days written notice. The Sponsor retains the right to terminate the study immediately upon written notice. If a clinical study is terminated early for whatever reason, the Investigator will return all samples, supplies and CRFs to the Sponsor and will notify the Main REC. Whichever party terminates the study will provide a written statement as to the reason for the termination. The Sponsor will notify the MHRA of premature terminations.

10.6 Compensation for medicine-induced injury and indemnification requirements.

Oxagen Ltd carries no fault clinical trial indemnity insurance, which is valid in the U.K. In the event of a proven medicine-induced injury, proof of guilt or negligence will not be required. Settlements will be decided by arbitration and the decision of the arbitrator will be final.

10.7 Study personnel

Principal Investigator: Trevor Hansel
Medical Director
National Heart and Lung Institute Clinical Studies Unit,
First Floor, Royal Brompton Hospital,
Fulham Road,
London SW3 6HP,
United Kingdom.
Telephone: 020 7351 8974
Facsimile: 020 7351 8973
Email: t.hansel@imperial.ac.uk

Study Sponsor: Oxagen Ltd
91 Milton Park
Abingdon
OXON

OX14 4RY
Authorised representative: Dr C Mike Perkins MD
Telephone: 01235 443300
Facsimile: 01235 443301
Email: m.perkins@oxagen.co.uk

Medical Monitor: Dr J Steiner
Oxford Therapeutics Consulting Ltd.,
Magdalen Centre,
The Oxford Science Park,
Oxford OX4 4GA
Telephone: 01865 784874
Facsimile: 01865 784875
Mobile: 07774 967074

Clinical Research Organisation:
Akos Limited,
The Coach House,
The Grove,
Piper's Lane,
Harpenden
Hertfordshire AL5 1AH
Telephone: 01582 766339
Facsimile: 01582 764327

Data Management and Statistical Analysis will be by:
Akos Limited,
The Coach House,
The Grove,
Piper's Lane,
Harpenden
Hertfordshire AL5 1AH
Telephone: 01582 766339
Facsimile: 01582 764327

10.8 Publication policy

Oxagen Ltd encourages publication of clinical trial data in reputable peer reviewed journals. Authorship will be discussed and agreed in advance. If the Investigator drafts a publication, he/she agrees to send it to Oxagen Ltd for review and comment before its submission to the journal. In cases where Oxagen considers that the proposed publication contains patentable material or information which should be protected as valuable confidential information, Oxagen Ltd reserves the right to delay submission to the journal until patent applications have been filed and/or require the deletion of the confidential information from the proposed publication.

10.9 Protocol amendments

All items in this protocol must be followed exactly. If any deviations occur, they must be documented and explained. If an amendment is required, this must be enacted through a formal documented protocol amendment procedure and must receive approval from all the authorities who approved the original protocol. The approved amendment will be distributed to all protocol recipients with instructions to append them to the protocol (or a revised protocol will be issued).

11. BIBLIOGRAPHY

1 Howarth PH, Persson CG, Meltzer EO, Jacobson MR, Durham SR, Silkoff PE. Objective monitoring of nasal airway inflammation in rhinitis. *J Allergy Clin Immunol* 2005;**115**:S414-S441.

2 Wang DY, Raza MT, Goh DY, Lee BW, Chan YH. Acoustic rhinometry in nasal allergen challenge study: which dimensional measures are meaningful? *Clin Exp.Allergy* 2004;**34**:1093-8.

3 Naclerio RM, Rosenwasser LJ, Ohkubo K. Allergic rhinitis: current and future treatments. *Clin Exp All Rev* 2002;**2**:137-47.

4 Barnes PJ. New drugs for asthma. *Nat.Rev Drug Discov.* 2004;**3**:831-44.

5 Chanez P, Vignola AM, Vic P, Guddo F, Bonsignore G, Godard P *et al.* Comparison between nasal and bronchial inflammation in asthmatic and control subjects. *Am J Respir Crit Care Med* 1999;**159**:588-95.

6 American Thoracic Society Workshop. Immunobiology of asthma and rhinitis. Pathogenic factors and therapeutic options. *Am J Respir Crit Care Med* 1999;**160**:1778-87.

7 Gelfand EW. Inflammatory mediators in allergic rhinitis. *J Allergy Clin Immunol* 2004;**114**:S135-S138.

8 Corren J. Allergic rhinitis and asthma: how important is the link? *J Allergy Clin Immunol* 1997;**99**:S781-S786.

9 Braunstahl GJ. The unified immune system: respiratory tract-nasobronchial interaction mechanisms in allergic airway disease. *J Allergy Clin Immunol* 2005;**115**:142-8.

10 Togias A. Rhinitis and asthma: Evidence for respiratory system integration. *J Allergy Clin Immunol* 2003;**111**:1171-83.

11 Bousquet J, Van Cauwenberge P, Khalttaev N, and the WHO Panel. Allergic rhinitis and its impact on asthma. ARIA. In collaboration with the World Health Organization. *J Allergy Clin Immunol* 2001;**108**:S1-S315.

12 Bousquet J, Jacquot W, Vignola AM, Bachert C, Van Cauwenberge P. Allergic rhinitis: a disease remodeling the upper airways? *J Allergy Clin Immunol* 2004;**113**:43-9.

13 Davies DE, Wicks J, Powell RM, Puddicombe SM, Holgate ST. Airway remodeling in asthma: new insights. *J Allergy Clin Immunol* 2003;**111**:215-25.

14 Larche M, Robinson DS, Kay AB. The role of T lymphocytes in the pathogenesis of asthma. *J Allergy Clin Immunol* 2003;**111**:450-63.

15 Zimmermann N, Hershey GK, Foster PS, Rothenberg ME. Chemokines in asthma: cooperative interaction between chemokines and IL-13. *J Allergy Clin Immunol* 2003;**111**:227-42.

16 Ono SJ, Nakamura T, Miyazaki D, Ohbayashi M, Dawson M, Toda M. Chemokines: Roles in leukocyte development, trafficking, and effector function. *J Allergy Clin Immunol* 2003;**111**:1185-99.

17 Sim TC, Reece LM, Hilsmeier KA, Grant JA, Alam R. Secretion of chemokines and other cytokines in allergen-induced nasal responses: inhibition by topical steroid treatment. *Am J Respir Crit Care Med* 1995;**152**:927-33.

18 Schmid-Grendelmeier P, Altznauer F, Fischer B, Bizer C, Straumann A, Menz G *et al.* Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 2002;**169**:1021-7.

19 Ghaffar O, Laberge S, Jacobson M, Lowhagen O, Rak S, Durham S *et al.* IL-13 mRNA and immunoreactivity in allergen-induced rhinitis:Comparison with IL-4 expression and modulation by topical glucocorticoid therapy. *Am J Respir Cell Mol Biol* 1997;**17**:17-24.

20 Romagnani S. The role of lymphocytes in allergic disease. *J Allergy Clin Immunol* 2000;**105**:399-408.

21 Greiff L, Petersen H, Mattsson E, Andersson M, Erjefalt JS, Linden M *et al.* Mucosal output of eotaxin in allergic rhinitis and its attenuation by topical glucocorticosteroid treatment. *Clin Exp Allergy* 2001;**31**:1321-7.

22 Ciprandi G, Tosca MA, Passalacqua G, Canonica GW. Intranasal mometasone furoate reduces late-phase inflammation after allergen challenge. *Ann.Allergy Asthma Immunol.* 2001;**86**:433-8.

23 Masuyama K, Till SJ, Jacobson MR, Kamil A, Cameron L, Juliusson S *et al.* Nasal eosinophilia and IL-5 m-RNA expression in seasonal allergic rhinitis induced by natural allergen exposure in seasonal allergic rhinitis induced by natural allergen exposure: effect of topicial steroids. *J Allergy Clin Immunol* 1998;**102**:610-7.

24 Linden M, Svensson C, Andersson E, Andersson M, Greiff L, Persson CG. Immediate effect of topical budesonide on allergen challenge-induced nasal mucosal fluid levels of granulocyte-macrophage colony-stimulating factor and interleukin-5. *Am.J Respir Crit Care Med.* 2000;**162**:1705-8.

25 Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr., Meyers DA, Norman PS *et al.* Mediator release after nasal airway challenge with allergen. *Am Rev.Respir Dis* 1983;**128**:597-602.

26 Greiff L, Pipkorn U, Alkner U, Persson CG. The 'nasal pool' device applies controlled concentrations of solutes on human nasal airway mucosa and samples its surface exudations/secretions. *Clin Exp Allergy* 1990;**20**:253-9.

27 Grunberg K, Timmers MC, Smits HH, de Klerk EP, Dick EC, Spaan WJ *et al.* Effect of experimental rhinovirus 16 colds on airway hyperresponsiveness to histamine and interleukin-8 in nasal lavage in asthmatic subjects in vivo. *Clin Exp.Allergy* 1997;**27**:36-45.

28 Belda J, Parameswaran K, Keith PK, Hargreave FE. Repeatability and validity of cell and fluid-phase measurements in nasal fluid: a comparison of two methods of nasal lavage. *Clin Exp Allergy* 2001;**31**:1111-5.

29 Pipkorn U, Proud D, Lichtenstein LM, Kagey-Sobotka A, Norman PS, Naclerio RM. Inhibition of mediator release in allergic rhinitis by pretreatment with topical glucocorticosteroids. *N.Engl.J Med.* 1987;**316**:1506-10.

30 Creticos PS, Peters SP, Adkinson NF, Jr., Naclerio RM, Hayes EC, Norman PS *et al.* Peptide leukotriene release after antigen challenge in patients sensitive to ragweed. *N Engl J Med* 1984;**310**:1626-30.

31 Naclerio RM, Proud D, Togias AG, Adkinson NF, Jr., Meyers DA, Kagey-Sobotka A *et al.* Inflammatory mediators in late antigen-induced rhinitis. *N Engl J Med* 1985;**313**:65-70.

32 Terada N, Hamano N, Kim WJ, Hirai K, Nakajima T, Yamada H *et al.* The kinetics of allergen-induced eotaxin level in nasal lavage fluid: its key role in eosinophil recruitment in nasal mucosa. *Am.J Respir Crit Care Med.* 2001;**164**:575-9.

33 Alam R, Sim TC, Hilsmeier K, Grant JA. Development of a new technique for recovery of cytokines from inflammatory sites in situ. *J Immunol Methods* 1992;**155**:25-9.

34 Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am.J Respir Crit Care Med.* 1994;**149**:339-44.

35 Weido AJ, Reece LM, Alam R, Cook CK, Sim TC. Intranasal fluticasone propionate inhibits recovery of chemokines and other cytokines in nasal secretions in allergen-induced rhinitis. *Ann.Allergy Asthma Immunol.* 1996;**77**:407-15.

36 Erin EM, Zacharasiewicz AS, Nicholson GC, Higgins LA, Tan AJ, Williams TJ *et al.* Topical corticosteroids inhibit IL-4, IL-5, and IL-13 levels in nasal secretions following local allergen challenge. *Clin Exp Allergy (In Press)* Dec 2005.

37 Erin EM, Leaker BR, Zacharasiewicz AS, Higgins LA, Williams TJ *et al.* Single dose topical corticosteroid inhibits IL-5 and IL-13 in nasal lavage following grass pollen challenge. *Allergy* 2005; 60:1524-29

38 Erin EM, Leaker BR, Zacharasiewicz AS, Tan AJ, Higgins LA, Boyce MJ *et al.* Effects of a reversible â-tryptase and trypsin inhibitor (RWJ-58643) on nasal allergic responses. *Clin Exp Allergy (In Press)* 2005.

39 Varney, V *et al.* Am. Rev. Respir. Dis. 146:170-176

12. INVESTIGATOR STATEMENT

I, the undersigned, have read and agree with protocol no OC000459/005/05, entitled:

"The effects of OC000459 (a CRTH2 antagonist) on nasal Th2 cytokine (IL-4/5/13) release, eosinophil responses, and nasal symptoms after nasal allergen challenge (NAC) with Timothy grass pollen in subjects with allergic rhinitis out of season: A double blind, randomised, two way crossover, placebo-controlled study in up to 20 subjects of the effect of a short course of OC000459 (400mg oral, OD with food am for 5 days)"

I agree that, in conducting this study, I shall comply with all the requirements of the protocol.

Signed

Name, title and position TREVOR HANSEL, MEDICAL DIRECTOR, NHLI CLINICAL STUDIES UNIT

Date 6th FEB. 2006

For and on behalf of National Heart and Lung Institute

Signed

Name, title and position HELEN NEIGHBOUR CLINICAL RESEARCH FELLOW NHLI CLINICAL STUDIES UNIT

Date 8/2/06

Signed

Name, title and position

Date

Signed

Name, title and position Dr C MIKE PERKINS MD, DEVELOPMENT DIRECTOR

Date 13th February 2006.

For and on behalf of Oxagen Ltd.

...X 1

...TICIPANT INFORMATION SHEET AND CONSENT FORM ADULT

...UNTEER

Before you decide to take part in this study, you must read this Information and Consent Form carefully and receive satisfactory answers to any questions that you might have. We would like you to take this form home and consider it carefully, before you decide whether or not to sign it. If you do participate, you should keep this form and refer to it during the study. Your participation is entirely voluntary and you will have unlimited time to decide whether or not to participate. The study will be conducted according to international Good Clinical Practice guidelines for clinical research.

TITLE OF PROJECT:

The effects of OC000459 (a CRTH2 antagonist) on nasal Th2 cytokine (IL-4/5/13) release, eosinophil responses, and nasal symptoms after nasal allergen challenge (NAC) with Timothy grass pollen in subjects with allergic rhinitis out of season:
A double blind, randomised, two way crossover, placebo-controlled study in up to 20 subjects of the effect of a short course of OC000459 (400mg oral, OD with food am for 5 days)

PROTOCOL NUMBER: **OC000459/005/05**

INTRODUCTION

We would like to invite you to participate in a research study. The study will be conducted at the National Heart and Lung Institute Clinical Studies Unit, Royal Brompton Hospital, Fulham Road, London SW3 6HP. This study has been reviewed and approved by the Brent Medical Ethics Committee.

What is the purpose of the study?

The drug being tested in this study is called OC000459. This is an experimental drug which is thought to have the potential to reduce the signs and symptoms of allergy and asthma. As an experimental drug in a clinical study, it means that the drug will only be given under the supervision of the staff of the Clinical Studies Unit, since this drug is not licensed for general use. If you choose to participate in this study, you will receive the OC000459 in the form of capsules for 5 days and also the placebo (a dummy substance which looks like the real thing but does not contain any active ingredient) for 5 days during two separate treatment periods which will be separated by a 2-3 week washout period.

The purpose of this study is to assess the effect OC000459 has on hormones and blood cells which move from your blood into the lining of your nose and the chemicals produced in an allergic response to a Timothy grass pollen. The pollen causes hayfever symptoms such as sneezing, itchy and running nose. ...e allergic response to Timothy grass pollen will be compared with a placebo. This will help to give us a ...st better understanding of allergic reactions such as hayfever and asthma.

Why have I been chosen?

You have been approached because you have a history of hayfever, and in particular, you have a sensitivity to Timothy grass pollen.

Do I have to take part?

No. Your participation in this study is voluntary. It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form. If you decide to take part you are still free to withdraw at any time and without giving a reason. A decision to withdraw at any time, or a decision not to take part, will not affect the standard of care you may receive at this hospital.

What will happen to me if I take part?

The study will involve a total of 7 visits over a period of 5-7 weeks, with a follow up visit 4 weeks later.

1 (Screening visit) : 2 hours

screening process will be carried out to find out if you are eligible for the study. During this visit you l have time to discuss the study with the study doctor and get answers to any questions that you may ave. You will then be invited to sign this consent form. You will undergo the following tests as part of the screening procedure:

Medical History: You will be asked about any medication you are taking and details of your allergies and other relevant medical history. Personal details (e.g. date of birth, next of kin, weight & height) will be taken.

Physical Examination: The study doctor will perform a general physical examination including careful examination of your heart and lungs. In addition, the physician will check your nose to make sure that your nasal structure is normal and your nasal passages are not blocked.

Skin prick test: At this time we will also perform a skin prick test to establish if you are sensitive to Timothy grass pollen. This involves placing a small amount of allergen extract, in this case Timothy grass pollen, on your forearm and introducing it into the surface layer of your skin with a sterile lancet. This can feel a little uncomfortable but should not be painful and does not draw blood. To confirm the test has worked properly a positive and negative control test will also be performed. With a positive reaction the skin becomes itchy within a few minutes and then becomes red with a "wheal" (lump) in the centre (very much like the reaction to a nettle sting), which will be measured after 15 minutes. This should go away within 30 minutes to an hour. Antihistamines will stop the test working properly, so should not be taken in the 48 hours before the test is performed.

Vital signs: Your blood pressure, pulse, body temperature and respiratory rate will be measured.

ECG: an electrocardiogram will be performed to get a tracing of your heart activity. You will be asked to lie down while electrodes are placed on your chest, arms and legs. This test is routine and painless.

Blood tests: About 10mls (2 teaspoons) of blood will be taken for routine safety tests.

Urine tests: You will be asked to provide a urine sample for routine safety tests and for a drugs of abuse screen.

Smoking test: You will be asked to breathe into a handheld machine that detects carbon monoxide in your breath. This is an indicator of whether you have inhaled cigarette smoke in the recent past.

Alcohol test: You will be asked to breathe into a handheld machine that detects alcohol in your breath. This is an indicator of whether you have drunk alcohol in the recent past. A positive result here will not exclude you from the study but if you take part in the study you will be asked to stop drinking alcohol from the evening before the start of and during each treatment period of 5 days.

Nasal symptoms: will be assessed by means of a questionnaire containing 4 short questions on nasal obstruction, runny nose (rhinorrhea), nasal itch and sneezing.

Nasal allergen challenge (NAC): a very minute quantity of Timothy grass pollen allergen is dissolved in a liquid and a droplet will be given via a small nasal pump spray into your right and left nostrils.

Nasal washings (also known as **nasal lavage):** For this procedure you will be asked to sit down, fit a "nasal olive" snugly into your nostril and bend your head and neck forward. The "nasal olive" is a small olive shaped plug with a hole through the middle through which 5ml (about a teaspoon) of a saline solution (salt water) will be gently flushed into and out of your nostril 20 times over a 1 minute period. The fluid obtained from nasal washings will be analysed for cells which causes your hayfever symptoms. Nasal washing is performed at screening (1 hour after the NAC) to ensure that there are enough cells in your nasal wash fluid to analyse using a microscope and to ensure that you are able to tolerate this procedure.

If you are suitable for the study, you will be invited to return to the clinical studies unit for the main study.

Visit 2 (Day 1 - Treatment Period 1) : 30 mins

Vital Signs: Your blood pressure and pulse, body temperature and respiratory rate will be measured.
Blood tests: About 10mls (2 teaspoons) of blood will be taken for routine safety tests.
Urine tests: You will be asked to provide a urine sample for routine safety tests and also for tests of drugs of abuse. A positive result for drugs would exclude you from the study.
Smoking test: You will be asked to breathe into a handheld machine that detects carbon monoxide in your breath. This is an indicator of whether you have inhaled cigarette smoke in the recent past. A positive result here would exclude you from the study.
Alcohol test: You will be asked to breathe into a handheld machine that detects alcohol in your breath. This is an indicator of whether you have drunk alcohol in the recent past. A positive result here would exclude you from the study.

At this visit you will be given 16 capsules of either the study drug or a placebo (inactive dummy substance) which you will take once a day in the morning at 8am after food. You will be asked to take the first 4 capsules on the unit during this visit, the next 12 will be taken at home by yourself (four capsules a day for the next 3 days). Four further capsules will be taken at your next visit to the unit (Visit 3). You will be given the study drug or placebo in a random and blinded order so that neither you nor the study doctor will know which treatment you are receiving. However the identity of the treatment is available to your doctor should this information be required. You will visit the unit 4 days later.

Visit 3 (Days 4 & 5 - Treatment Period 1): 24 hrs

During this visit you will be asked to report to the Clinical Studies Unit at about 9.00 p.m. on the fourth day of dosing. We would like you to have had your supper or dinner prior to arrival on the unit. You will spent the night on the unit where we have sleeping and shower facilities. Please bring an overnight bag consisting of a toothbrush, shaving kit, soap, comfortable clothing, books and magazines to read during your stay with us. Beverages, toasts, biscuits and meals will be provided. For your own safety, we would like you to remain on the unit until about 9pm the following evening.
Urine tests: You will be asked to provide a urine sample for tests of drugs of abuse. A positive result for drugs would remove you from the study.
Smoking test: You will be asked to breathe into a handheld machine that detects carbon monoxide in your breath. This is an indicator of whether you have inhaled cigarette smoke in the recent past. A positive result here would remove you from the study.
Alcohol test: You will be asked to breathe into a handheld machine that detects alcohol in your breath. This is an indicator of whether you have drunk alcohol in the recent past. A positive result here would remove you from the study.
At 6.30 a.m. on the next morning, you will have blood and urine safety tests taken and your vital signs will be checked (as Day 1 above). After this you will have a standard full fat breakfast and then you will be asked to take the last four capsules at 7am. The nasal allergen challenge will be done three hours after the dose of capsules. The following procedures will be performed:
Blood tests: about 10mls (2 teaspoons) of blood will be taken for routine safety tests in the morning (as mentioned above) and in the evening. You will also be asked to provide a urine sample for safety tests at each time point.
Tests of your heart rate and blood pressure.
Blood tests of the amount of drug in your blood (six samples of 2 ml each).
Nasal allergen challenge: a very small quantity of the Timothy grass pollen, an allergen which causes you to have hayfever symptoms will be administered into both your nostrils via a nasal pump spray.
Nasal symptoms: will be assessed 15 min before and following the allergen administration at 30min, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours. We will record nasal symptoms by means of a questionnaire.
Nasal filter paper placement: This technique has been developed to provide a non-invasive method of sampling relatively undiluted chemicals present in the fluid lining the nose of hayfever sufferers. These chemicals partly control the movement of blood cells from the blood into the nose in hayfever. 2 strips (size 7mm wide x 30 mm long) of filter paper will be placed on the inner surface of your right nostril for 2 minutes

absorb fluid from the lining of your nose. After 2 minutes the filter paper will be removed for analysis of these chemicals. This procedure will be carried out 15mins preceding the nasal allergen challenge and then following nasal allergen administration at 15min, 30min, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours.

Nasal washings: Nasal washings in the nose will be carried out by passing a small volume of saline fluid into the nose, and then the fluid is gently flushed into and out of your left nostril 20 times over a 1 minute period. This procedure will be carried out 15 min before and following the allergen administration at 30min, 1, 2, 4, 6 and 8 hours. The fluid obtained from the nasal washings will be analysed for cells as described above.

Visit 4 (Day 6 - Treatment period 1 - Safety questions) : 10mins

You will be telephoned by the Clinical Studies Unit the following morning to ask about any symptoms you may have experienced over the 12 hours since leaving the CSU and to ask about any changes that you may have made to any medications that you may have been taking.

The first treatment period will be followed by an interval of 14 - 21 days.

Visit 5 (Day 1 - Treatment period 2) : 30mins

Vital Signs: Your blood pressure and pulse, body temperature and respiratory rate will be measured.

ECG: an electrocardiogram will be performed to get a tracing of your heart activity.

Blood tests: About 10mls (2 teaspoons) of blood will be taken for routine safety tests.

Urine tests: You will be asked to provide a urine sample for routine safety tests and also for tests of drugs of abuse. A positive result for drugs would exclude you from the study.

Smoking test: You will be asked to breathe into a handheld machine that detects carbon monoxide in your breath. This is an indicator of whether you have inhaled cigarette smoke in the recent past. A positive result here would exclude you from the study.

Alcohol test: You will be asked to breathe into a handheld machine that detects alcohol in your breath. This is an indicator of whether you have drunk alcohol in the recent past. A positive result here would remove you from the study.

You will also be given 16 capsules of either the study drug or a placebo (inactive dummy substance) which you will take once a day in the morning at 8am after food. You will be asked to take the first 4 capsules on the unit during this visit, the next 12 will be taken at home by yourself (four capsules a day for the next 3 days). Four further capsules will be taken at your next visit to the unit (Visit 6). You will be given the study drug or placebo in a random and blinded order so that neither you nor the study doctor will know which treatment you are receiving. However the identity of the treatment is available to your doctor should the information be required. You will visit the unit 4 days later.

Visit 6 (Days 4 & 5 Treatment Period 2) : 24hrs

During this visit you will be asked to report to the Clinical Studies Unit at about 9.00 p.m. on the fourth day of dosing. We would like you to have had your supper or dinner prior to arrival on the unit. You will spent the night on the unit and we would like you to bring an overnight bag as discussed above. For your own safety, we would like you to remain on the unit until about 9pm the following evening.

At 6.30 a.m. on the next morning, you will have blood and urine tests taken and your vital signs will be checked. After this you will have a standard full fat breakfast and then you will be asked to take the last four capsules at 7am. The nasal allergen challenge will be done three hours after the dose of capsules. The following procedures will be performed:

Blood tests: about 10mls (2 teaspoons) of blood will be taken for routine safety tests in the morning (as mentioned above) and in the evening. You will also be asked to provide a urine sample for safety tests at each time point.

Tests of your heart rate and blood pressure.

Blood tests of the amount of drug in your blood (six samples of 2 ml each).
Nasal allergen challenge: a very small quantity of the Timothy grass pollen, an allergen which causes you to have hayfever symptoms will be administered into both your nostrils via a nasal pump spray.
Nasal symptoms: will be assessed 15 min before and following the allergen administration at 30min, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours. We will record nasal symptoms by means of a questionnaire.
Nasal filter paper placement: 2 strips (size 7 x 30 mm/strip) of filter paper will be placed on the inner surface of your right nostril for 2 minutes to absorb fluid from the lining of your nose. After 2 minutes the filter paper will be removed for analysis as described above. This procedure will be carried out 15mins preceding the nasal allergen challenge and then following nasal allergen administration at 15min, 30min, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours.
Nasal washings: Nasal washings in the nose will be carried out by passing a small volume of saline fluid into the nose, and then the fluid is gently flushed into and out of your left nostril 20 times over a 1 minute period. This procedure will be carried out 15 min before and following the allergen administration at 30min, 1, 2, 4, 6 and 8 hours. The fluid obtained from the nasal washings will be analysed for cells as described above.

Visit 7 (Day 6 - Treatment Period 2 - Safety questions) : 10mins

You will be telephoned by the Clinical Studies Unit the following morning to ask about any symptoms you may have experienced over the 12 hours since leaving the CSU and to ask about any changes that you may have made to any medications that you may have been taking.

Visit 8 (Follow Up after 4 weeks) : 1hr
At the follow-up visit, we will carry out some of the same procedures as described at visit 1 as a safety precaution:
Physical Examination: A general physical examination will be performed by the study doctor.
Vital Signs: Your blood pressure and pulse, body temperature and respiratory rate will be measured.
ECG: an electrocardiogram will be performed to get a tracing of your heart activity.
Blood tests: about 10mls (2 teaspoons) of blood will be taken for routine safety tests. You will also be asked to provide a urine sample for safety tests.

What are the potential discomforts and risks of taking part?
Risks from study drug: No severe side effects have been demonstrated in human studies to date (about 70 healthy volunteers have been studied). Animal tests indicate that there may be an effect of blood sugar but this has not been shown in man.

Timothy grass pollen allergen has been safely administered to the nose of volunteers with hayfever in previous studies at the National Heart and Lung Institute. There were only minor symptoms of nasal itch and local symptoms of clear discharge (runny nose), sneezing, and nasal blockage but these effects were short-lived.

There is also a small risk of an anaphylactic reaction (a severe allergic reaction which is potentially life threatening) secondary to nasal allergen challenge for which emergency provisions are in place on the Clinical Studies Unit. No such reaction has taken place on the Clinical Studies Unit in the past 10 years with inhaled and nasal allergen challenge.

It is thought that through repeated nasal allergen challenges the reaction to the challenge and the hayfever symptoms may increase. If the symptoms become too uncomfortable for you (or your doctor thinks the symptoms are too severe) you will be given medication to help with the symptoms and will be discontinued from the study.

Nasal washing and filter paper placement may be associated with mild discomfort but there are no long-term effects.

ing a blood sample may cause mild to moderate pain, and may result in temporary bruising to your arm. otal of 120mls (about 8 tablespoons) will be taken for this study.

***Note:* All specimens (blood, cell samples etc) that are obtained from you will be used only for the specified tests and then destroyed.**

Are there any study restrictions I should be made aware of?
Prescribed medications are not allowed for the two weeks prior to your inclusion in the study or for the duration of the study. You will also be asked not to take any over the counter medicines (including vitamins), especially non-steroidal anti-inflammatory drugs (e.g. ibuprofen), for the same time period.
You must not have smoked during the last year nor smoke during the study, nor have used any drugs of abuse recently or during the study. You will also not be allowed to drink alcohol during the two treatment periods (5 days each), starting from the evening before you attend the Clinical Studies Unit for each treatment period. Tests will be done for smoking, alcohol and drugs of abuse at regular intervals during the study. You will also be asked not to drink caffeine containing drinks (e.g. tea, coffee, cola) nor to exercise during these two 5 day treatment periods.
You will be required to eat identical full fat breakfasts on day 5 of each of the two treatment periods before taking your capsules that morning. These breakfasts are likely to consist of baked beans, hash browns, rk sausages, omelette, all butter croissants, butter, jam and a drink (juice or decaffeinated tea or coffee).
ssr
The capsules containing study medication will be made using gelatine.

What will happen to any samples I give?
All specimens (blood, cell samples etc) that are obtained from you will be used only for the specified tests and then destroyed. All samples obtained from this study will also be labelled in such a way which will make it impossible to identify you personally. Only relevant personnel from the NHLI Clinical Studies Unit and BioDynamics will have access to these samples.

What do I have to do?
You will be asked to attend all scheduled visits at the times and for the duration specified and you must be willing to participate in all the procedures as described above.
You will be asked not to participate in another clinical trial or donate blood or take any medications other than paracetamol whilst participating in this study. If you have to take medications for whatever reasons, please ring and let the study doctor know before you do so.

What are the potential benefits of taking part?
Your participation in this study will not benefit you directly. Indirect benefits may include the possible advancement of medical knowledge so that scientists can find more effective and safer treatments for hay
as ver and allergic diseases.

What if relevant new information becomes available?
Sometimes during the course of a research project, new information may become available which may be relevant to you. If this happens, your research doctor will tell you about it and discuss whether you want to or should continue in the study. If you decide not to carry on, your research doctor will make arrangements for your care to continue. If you decide to continue in the study you will be asked to sign an updated consent form.

Also, on receiving new information your research doctor might consider it to be in your best interests to withdraw you from the study. He/she will explain the reasons and arrange for your care to continue.

If the study is stopped for any other reason, you will be told why and your continuing care will be arranged.

...at if something goes wrong?
...the very remote event of anything going wrong, we also have arrangements for you to receive ...ompensation, through our Imperial College insurance scheme and through Oxagen, the sponsor of this trial. Imperial College and Oxagen hold Public Liability ("negligent harm") and Clinical Trial ("non-negligent harm") insurance policies which apply to this trial. If you can demonstrate that you experienced harm or injury as a result of your participation in this trial, you will be eligible to claim compensation without having to prove that Imperial College or Oxagen is at fault. If the injury resulted from any procedure which is not part of the trial, Imperial College will be required to compensate you in this way. Your legal rights to claim compensation for injury where you can prove negligence are not affected.

Will my taking part in this study be kept confidential?
Your name will not be disclosed outside of the clinic. To make sure the information collected in the study is accurate, it will need to be checked by researchers working for or acting on behalf of Oxagen and possibly by members of the local ethics committee and government health departments. You are asked to give permission for these researchers to see your medical records. They will keep the information confidential.

Will my GP be informed of my involvement in this study?
Your GP will be informed of your participation in this study.

What will happen to the results of the research study?
...ssie results of this research may be presented at scientific meetings or in publications, but your identity will not be disclosed. Should you wish to see the results of the study, your study doctor will be able to help you. We ask you to waive any commercial rights which may arise from the study.

Will I get paid for participating in this study?
You will be reimbursed up to £700 for your travelling and out of pocket expenses incurred by participating in this study. If you are withdrawn from the study for medical reasons you will be reimbursed up to the point of withdrawal.

Who is funding this research?
The costs of this research study are being sponsored by a pharmaceutical company called Oxagen Ltd.

Who can I contact if I have any questions about this study?
We would like you to be fully informed about the nature of this study, your rights as a trial subject, and whom to contact in the event of trial-related injury. You should not hesitate to report any effect that is upsetting you, and you must contact the Clinical Studies Unit immediately if you feel unwell. You can reach a doctor on 020 7351 8971 during the week in office hours or on a mobile number which will be given to you upon commencement of the trial.

...r independent advice on participating in clinical trials, you may wish to speak to someone at the Royal Brompton Hospital's Patient Advice and Liaison Services (PALS) office on tel: 020 7352 8121.

If you have any complaints to make about your experience with the research, we would encourage you to speak to Dr Trevor Hansel (tel: 020 7351 8974) who the Principal Investigator of this study.

Thank you for taking time to read this form and for considering taking part in this study. You should not sign this consent form if you do not wish to participate in this research study.