APOLLO

**A P**roof-of-Concept Study for Vitamin A Nasal Drops in Post-Viral **Ol**factory **Lo**ss

|  |  |
| --- | --- |
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| Trial registration | [Trial registry name and identifier] |
|  |  |
| REC Reference# | [insert MREC number] |

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Table of Contents

[1 Administrative information 1](#_Toc65798873)

[1.1 Compliance 1](#_Toc65798874)

[1.2 Sponsor 1](#_Toc65798875)

[1.3 Structured trial summary 2](#_Toc65798876)

[1.4 Roles and responsibilities 5](#_Toc65798877)

[1.4.1 Protocol contributors 5](#_Toc65798878)

[1.4.2 Role of trial sponsor and funders 5](#_Toc65798879)

[1.4.3 Trial Team 5](#_Toc65798880)

[1.4.4 Study Management Group 6](#_Toc65798881)

[1.4.5 Independent Study Steering Committee 6](#_Toc65798882)

[2 Trial Diagram 7](#_Toc65798883)

[3 Abbreviations 8](#_Toc65798884)

[4 Glossary 9](#_Toc65798885)

[5 Introduction 10](#_Toc65798886)

[5.1 Background and Rationale 10](#_Toc65798887)

[The epidemiology and impact of olfactory disorders 10](#_Toc65798888)

[Current standard of care for PVOL 11](#_Toc65798889)

[Pathophysiology of olfactory dysfunction and rationale for objective assessments 11](#_Toc65798890)

[5.1.1 Explanation for choice of comparators 12](#_Toc65798891)

[5.2 Objectives 13](#_Toc65798892)

[5.3 Trial Design 14](#_Toc65798893)

[6.1 Site Selection 15](#_Toc65798894)

[6.1.1 Study Setting 15](#_Toc65798895)

[6.1.2 Site/Investigator Eligibility Criteria 15](#_Toc65798896)

[6.1.2.1 Principal Investigator’s (PI) Qualifications and Agreements 15](#_Toc65798897)

[6.1.2.2 Resourcing at site 15](#_Toc65798898)

[6.2 Site approval and activation 15](#_Toc65798899)

[6.3 Participants 16](#_Toc65798900)

[6.3.1 Eligibility Criteria 16](#_Toc65798901)

[6.3.1.1 Participant selection 16](#_Toc65798902)

[6.3.1.2 Participant Inclusion Criteria 16](#_Toc65798903)

[6.3.1.3 Participant Exclusion Criteria 16](#_Toc65798904)

[6.3.1.5 Co-enrolment Guidance 17](#_Toc65798905)

[6.3.1.6 Screening Procedures and Pre-randomisation Investigations 17](#_Toc65798906)

[6.4 Interventions 17](#_Toc65798907)

[6.4.1 Arm A 17](#_Toc65798908)

[6.4.1.1 Products 17](#_Toc65798909)

[6.4.1.2 Treatment Schedule 18](#_Toc65798910)

[6.4.1.3 Dispensing 18](#_Toc65798911)

[6.4.1.4 Dose Modifications, Interruptions and Discontinuations 18](#_Toc65798912)

[6.4.2 Arm B 18](#_Toc65798913)

[6.4.2.1 Products 18](#_Toc65798914)

[6.4.2.2 Treatment Schedule 18](#_Toc65798915)

[6.4.2.3 Dispensing 18](#_Toc65798916)

[6.4.2.4 Dose Modifications, Interruptions and Discontinuations 18](#_Toc65798917)

[6.4.3 Accountability 18](#_Toc65798918)

[6.4.4 Compliance and Adherence 18](#_Toc65798919)

[6.4.5 Concomitant Care 18](#_Toc65798920)

[6.4.6 Overdose of Trial Medication 19](#_Toc65798921)

[6.4.7 Protocol Treatment Discontinuation 19](#_Toc65798922)

[6.5 Outcomes 19](#_Toc65798923)

[6.5.1 Primary Outcomes 19](#_Toc65798924)

[6.5.2 Secondary Outcomes 19](#_Toc65798925)

[MRI scan OBV and sulcus measurements and fMRI changes 19](#_Toc65798926)

[Psychophysical smell test score and ODQ 20](#_Toc65798927)

[6.6 Participant Timeline 20](#_Toc65798928)

[6.6.1 Patient Assessments 21](#_Toc65798929)

[6.6.3 Early Stopping of Follow-up 21](#_Toc65798930)

[6.6.4 Participant Transfers 22](#_Toc65798931)

[6.6.5 Loss to Follow-up 22](#_Toc65798932)

[6.6.6 Trial Closure 22](#_Toc65798933)

[6.7 Sample Size 22](#_Toc65798934)

[6.8 Recruitment and Retention 22](#_Toc65798935)

[6.8.1 Recruitment 22](#_Toc65798936)

[6.8.2 Retention 23](#_Toc65798937)

[6.9 Assignment of Intervention 23](#_Toc65798938)

[6.9.1 Allocation 23](#_Toc65798939)

[6.9.1.1 Sequence generation 23](#_Toc65798940)

[6.9.1.2 Allocation concealment mechanism 23](#_Toc65798941)

[6.9.1.3 Allocation Implementation 23](#_Toc65798942)

[6.9.2 Blinding 23](#_Toc65798943)

[6.9.3 Emergency Unblinding 23](#_Toc65798944)

[6.10 Data Collection, Management and Analysis 23](#_Toc65798945)

[6.10.1 Data Collection Methods 23](#_Toc65798946)

[6.10.2 Data Management 24](#_Toc65798947)

[6.10.3 Non-Adherence and Non-Retention 25](#_Toc65798948)

[6.10.4 Statistical Methods 25](#_Toc65798949)

[6.10.4.1 Outcomes 25](#_Toc65798950)

[6.10.4.2 Statistical Analysis Plan 26](#_Toc65798951)

[6.10.4.3 Additional Analyses 26](#_Toc65798952)

[6.10.4.4 Analysis Population 26](#_Toc65798953)

[6.10.4.5 Missing Data 26](#_Toc65798954)

[6.11 Data Monitoring 26](#_Toc65798955)

[6.11.1 Data Monitoring Oversight 26](#_Toc65798956)

[6.11.2 Interim Analyses 26](#_Toc65798957)

[6.11.3 Data Monitoring for Harm 27](#_Toc65798958)

[6.11.3.1 Safety reporting 27](#_Toc65798959)

[6.11.3.2 Other Notifiable Adverse Events 28](#_Toc65798960)

[6.11.3.3 Exempted Adverse Events 28](#_Toc65798961)

[6.11.3.4 Procedures to follow in the event of female participants becoming pregnant 28](#_Toc65798962)

[6.11.3.5 Investigator responsibilities relating to safety reporting 28](#_Toc65798963)

[6.11.3.5.1 Seriousness assessment 28](#_Toc65798964)

[6.11.3.5.2 Severity or grading of Adverse Events 28](#_Toc65798965)

[6.11.3.5.3 Causality 28](#_Toc65798966)

[6.11.3.5.4 Expectedness 29](#_Toc65798967)

[6.11.3.6 Notifications 30](#_Toc65798968)

[6.11.3.6.1 Notifications by the Investigator to NCTU 30](#_Toc65798969)

[6.11.3.6.2 NCTU responsibilities 30](#_Toc65798970)

[6.11.4 Quality Assurance and Control 31](#_Toc65798971)

[6.11.4.1 Risk Assessment 31](#_Toc65798972)

[6.11.4.2 Central Monitoring at NCTU 31](#_Toc65798973)

[6.11.4.3 On-site Monitoring 31](#_Toc65798974)

[6.11.4.3.1 Direct access to participant records 31](#_Toc65798975)

[6.11.4.4 Trial Oversight 31](#_Toc65798976)

[6.11.4.4.1 Study Team 32](#_Toc65798977)

[6.11.4.4.2 Study Management Group 32](#_Toc65798978)

[6.11.4.4.3 Independent Study Steering Committee 32](#_Toc65798979)

[6.11.4.4.5 Trial Sponsor 32](#_Toc65798980)

[7 Ethics and Dissemination 34](#_Toc65798981)

[7.1 Research Ethics and Health Research Authority Approval 34](#_Toc65798982)

[7.2 Competent Authority Approvals 34](#_Toc65798983)

[7.3 Other Approvals 34](#_Toc65798984)

[7.4 Amendments 34](#_Toc65798985)

[7.5 Consent or Assent 34](#_Toc65798986)

[7.6 Confidentiality 35](#_Toc65798987)

[7.7 Declaration of Interests 35](#_Toc65798988)

[7.8 Indemnity 35](#_Toc65798989)

[7.9 Finance 35](#_Toc65798990)

[7.10 Archiving 35](#_Toc65798991)

[7.11 Access to Data 35](#_Toc65798992)

[7.12 Ancillary and Post-trial Care 36](#_Toc65798993)

[7.13 Publication Policy 36](#_Toc65798994)

[7.13.1 Trial Results 36](#_Toc65798995)

[Projected outputs: 36](#_Toc65798996)

[Expected research outputs: 36](#_Toc65798997)

[7.13.2 Authorship 36](#_Toc65798998)

[7.13.3 Reproducible Research 36](#_Toc65798999)

[8 Protocol Amendments 38](#_Toc65799000)

[9 References 39](#_Toc65799001)

[10 Appendices 43](#_Toc65799002)

[Appendix 1: SmPC for Vitamin A 43](#_Toc65799003)

[11 Principal Investigator compliance statement 49](#_Toc65799004)

# 1 Administrative information

This document was constructed using the Norwich Clinical Trials Unit (NCTU) Protocol template Version 4. It describes the APOLLO trial, sponsored by University of East Anglia and co-ordinated by NCTU.

It provides information about procedures for entering participants into the trial, and provides sufficient detail to enable: an understanding of the background, rationale, objectives, trial population, intervention, methods, statistical analyses, ethical considerations, dissemination plans and administration of the trial; replication of key aspects of trial methods and conduct; and appraisal of the trial’s scientific and ethical rigour from the time of ethics approval through to dissemination of the results. The protocol should not be used as an aide-memoire or guide for the treatment of other patients. Every care has been taken in drafting this protocol, but corrections or amendments may be necessary. These will be circulated to registered investigators in the trial. Sites entering participants for the first time should confirm they have the correct version through a member of the trial team at NCTU.

NCTU supports the commitment that its trials adhere to the SPIRIT guidelines. As such, the protocol template is based on the Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2012 Statement for protocols of clinical trials1. The SPIRIT Statement Explanation and Elaboration document2 can be referred to, or a member of NCTU Protocol Review Committee can be contacted for further detail about specific items.

## 1.1 Compliance

The trial will be conducted in compliance with the approved protocol, the Declaration of Helsinki (2008), the principles of Good Clinical Practice (GCP) as laid down by the Commission Directive 2005/28/EC with implementation in national legislation in the UK by Statutory Instrument 2004/1031 and subsequent amendments, the UK Data Protection Act, and the UK Policy Framework for Health and Social Care Research. Agreements that include detailed roles and responsibilities will be in place between participating sites and NCTU.

Participating sites will inform NCTU as soon as they are aware of a possible serious breach of compliance, so that NCTU can fulfil its requirement to report the breach if necessary, within the timelines specified in the UK Clinical Trials Regulations (currently 7 days). For the purposes of this regulation a ‘serious breach’ is one that is likely to affect to a significant degree:

* The safety or physical or mental integrity of the subjects in the trial, or
* The scientific value of the trial.

## 1.2 Sponsor

The University of East Anglia, represented by the Research and Innovation team (RIN), is the trial sponsor and has delegated responsibility for the overall management of the APOLLO trial activities to the Chief Investigator and NCTU. Queries relating to sponsorship of this trial should be addressed to RIN whereas queries in relation to trial activities should be address to the Chief Investigator or via the trial team.

## 1.3 Structured trial summary

|  |  |
| --- | --- |
| Primary Registry and Trial Identifying Number | ISRCTN registry 39523 – TBC once REC allocated |
| Date of Registration in Primary Registry | 23rd February 2021 – to be updated |
| Secondary Identifying Numbers | IRAS Number: 294741 |
| Source of Monetary or Material Support | National Institute for Health Research – Research for Patient Benefit Funding Stream |
| Sponsor | University of East Anglia |
| Contact for Public Queries | [NorwichCTU@uea.ac.uk](mailto:NorwichCTU@uea.ac.uk) |
| Contact for Scientific Queries | Prof. Carl Philpott  Norwich Medical School, UEA, NR4 7TJ  Tel: 01603 591105  C.Philpott@uea.ac.uk |
| Short Title or Acronym | APOLLO Proof of Concept Study |
| Scientific Title | **A P**roof-of-Concept Study for Vitamin A Nasal Drops in Post-Viral **Ol**factory **Lo**ss (APOLLO) |
| Countries of Recruitment | UK |
| Health Condition(s) or Problem(s) Studied | Postinfectious or post-viral olfactory loss/dysfunction (PVOL) |
| Intervention(s) | Intervention: Vitamin A drops (Vitadral Aristo Pharma GmbH, Berlin, Germany) self-administered intranasally via a dropper at a dose of 10,000 IU once daily for 12 weeks (2 drops per nostril)  Control: Peanut oil drops for the placebo arm will be administered in the same way for the same duration. |
| Key Inclusion and Exclusion Criteria | Inclusion Criteria:  A partial or total loss of smell due to post-viral olfactory loss as confirmed on history, examination and with a smell test (TDI) score of <31/48 and within 3 years of the precipitating viral infection.  Exclusion Criteria:   1. Participants with a history of:    1. chronic rhinosinusitis with/without nasal polyposis    2. severe nasal septal deviation    3. Major prior head injury    4. congenital olfactory loss    5. use of concurrent intranasal medications or possible medications know to affect olfaction    6. chronic renal disease    7. chronic hepatic disease    8. allergy to peanuts, soy or vitamin A (drops contain peanut oil) 2. Significant medical, surgical or psychiatric disease that in the opinion of the CI would affect subject safety or influence the study outcomes 3. Currently taking oral vitamin A supplements, anticoagulants or tetracyclines 4. Age of less than 18 5. Pregnant women and women of a childbearing age not using an effective contraceptive 6. Participants unsuitable for MRI due to metal implants, such as pacemaker etc, as is standard for MRI scanning or who move excessively during scanning. 7. Evidence from endoscopy or the initial MRI scan of:    1. Participants with any endoscopic findings of:       1. Chronic rhinosinusitis with/without nasal polyposis       2. Severe nasal septal deviation (preventing passage of 4mm endoscope)       3. Other inflammatory sinonasal disease    2. Participants with MRI changes indicating oedema in the sinuses and/or olfactory clefts 8. Any participant with a combined OBV of >85mm3 will be excluded as it is unlikely they will demonstrate significant increase in overall volume based on previous studies of OBV. 9. Participation in another clinical trial in the previous 4 months |
| Study Type | Mechanistic, proof of concept, double blind, placebo controlled RCT of Vitamin A Nasal Drops in Post-Viral Olfactory Loss |
| Date of First Enrolment | 15th September 2021. |
| Target Sample Size | Fifty-seven participants. |
| Primary Outcome(s) | Primary outcome measure: Olfactory bulb volume (on MRI scan) at 3 months |
| Key Secondary Outcomes | 1. Right orbital sulcus volume (MRI scan) 2. Blood-oxygen- level-dependant (BOLD) -signal in primary olfactory areas; amygdala, piriform, and insula (fMRI scan) 3. Psychophysical smell test (TDI) score 4. Olfactory disorders questionnaire (ODQ) score 5. Brain-derived neurotrophic factor level   All measures are at 3 months |

## 1.4 Roles and responsibilities

These membership lists are correct at the time of writing; please see terms of reference documentation in the TMF for current lists.

### 1.4.1 Protocol contributors

|  |  |  |
| --- | --- | --- |
| Name | Affiliation | Role |
| Professor Carl Philpott | UEA | CI, Lead author |
| Dr Sara Bengtsson (UEA) | UEA | Co-author |
| Dr Saber Sami | UEA | Co-author |
| Dr Allan Clark | UEA | Co-author |
| Professor Thomas Hummel | Technical University, Dresden | Co-author |
| Mr James Boardman | Fifth Sense | Co-author |

### 1.4.2 Role of trial sponsor and funders

|  |  |  |
| --- | --- | --- |
| Name | Affiliation | Role |
| Tracy Moulton | UEA | Sponsor representative |
| Nishita Nair | NIHR | Funding body representative |

### 1.4.3 Trial Team

|  |  |  |
| --- | --- | --- |
| Name | Affiliation | Role and responsibilities |
| Professor Carl Philpott | UEA | CI |
| Dr Sara Bengtsson | UEA | Lead on functional imaging |
| Dr Saber Sami | UEA | Lead on multi-modal imaging |
| Dr Allan Clark | UEA | Trial statistician |
| Professor Thomas Hummel | Technical University, Dresden | Expert advisor and CI of previous study of topical vitamin A in PVOL |
| Mr James Boardman | Fifth Sense | Patient expert/representative |

### 1.4.4 Study Management Group

|  |  |  |
| --- | --- | --- |
| Name | Affiliation | Role and responsibilities |
| Professor Carl Philpott | UEA | CI |
| Dr Sara Bengtsson | UEA | Lead on functional imaging |
| Dr Saber Sami | UEA | Lead on multi-modal imaging |
| Dr Allan Clark | UEA | Trial statistician |
| RA - TBC | UEA | Research Associate |

### 1.4.5 Independent Study Steering Committee

|  |  |  |
| --- | --- | --- |
| Name | Affiliation | Role and responsibilities |
|  |  |  |
|  |  |  |
|  |  |  |
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# 2 Trial Diagram

Baseline visit:

Complete ODQ; smell test if not done already

Consent and randomisation

# 3 Abbreviations

|  |  |
| --- | --- |
| AE | Adverse Event |
| AR | Adverse Reaction |
| BOLD | Blood oxygen level dependant |
| CI | Chief Investigator |
| CRF | Case Report Form |
| CRS | Chronic Rhinosinusitis |
| DMC | Data Management Committee |
| DTI | Diffusion Tensor Imaging |
| EU | European Union |
| FDA | (US) Food and Drug Administration |
| FWA | Federal Wide Assurance |
| GCP | Good Clinical Practice |
| HRA | Health Research Authority |
| ICH | International Conference on Harmonisation |
| IMP | Investigational Medicinal Product |
| ITT | Intention to Treat |
| JPUH | James Paget University Hospital NHS Foundation Trust |
| MHRA | Medicines and Healthcare products Regulatory Agency |
| (d)MRI | (diffusion) Magnetic Resonance Imaging |
| (f)MRI | (functional) Magnetic Resonance Imaging |
| NAE | Notifiable Adverse Event |
| NCTU | Norwich Clinical Trials Unit |
| NNUH | Norfolk and Norwich University Hospitals NHS Foundation Trust |
| OBV | Olfactory bulb volume |
| ODQ | Olfactory disorders questionnaire |
| OFC | Orbitofrontal cortex |
| ORN | Olfactory Receptor Neuron |
| PI | Principal Investigator |
| PID | Participant IDentification number |
| PIS | Participant Information Sheet |
| PROMS | Patient Reported Outcome Measures |
| PVOL | Post-viral olfactory loss |
| QA | Quality Assurance |
| QC | Quality Control |
| QMMP | Quality Management and Monitoring Plan |
| RA | Research Associate |
| RAc | Retinoic Acid |
| R&D | Research and Development |
| REC | Research Ethics Committee |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SAR | Serious Adverse Reaction |
| SPC | Summary of Product Characteristics |
| SSA | Site Specific Approval |
| SSC | Study Steering Committee |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| TDI | Threshold, Discrimination, Identification |
| TMF | Trial Master File |
| TMG | Trial Management Group |
| TMT | Trial Management Team |
| ToR | Terms of Reference |
| TSC | Trial Steering Committee |
| TNSTC | The Norfolk Smell & Taste Clinic |
| UADE | Unexpected Adverse Device Effect |
| UBIC | UEA Brain Imaging Centre |
| UEA | University of East Anglia |

# 4 Glossary

None

# 5 Introduction

## 5.1 Background and Rationale

### The epidemiology and impact of olfactory disorders

Loss of smell is a common complaint in adults and yet its impact has been underestimated. Anosmia, complete loss of smell, is thought to affect at least 1% of the global population with the overall estimated prevalence of olfactory disorders being between 1 and 20% 3-6. Based on European estimates, anosmia is more prevalent than profound hearing loss or blindness in the UK 7. Recent evidence from several population studies show8 that it is anosmia and not hearing or sight loss, that is an independent risk factor for a shortened life span9-12 with this sensory loss acting as a marker of cumulative toxic environmental exposures13. Causes for olfactory loss are varied but the main diagnostic groups include sinonasal disease (62%) and post-viral olfactory loss (PVOL) (11%)14,15. There is now an emerging new cluster of patients with PVOL – those infected with the SARS-CoV-2 virus as part of the global pandemic. With smell loss now an official World Health Organisation symptom of Covid-19, evidence has clearly shown that over 60% of those contracting the virus are affected by this symptom16,17. This means that over 1 billion people have experienced smell loss due to Covid-19 globally to date. The majority of those affected appear to recover their sense of smell within 4 weeks of the onset, but current data suggests that 10-17% have continued smell loss and do not recover spontaneously18,19; based on the UK infection rate (20th Apr 2021), this means an estimated 250,000 people now have PVOL due to Covid-19.

There is wide variation in clinical practice and little consistent information provided by clinicians to patients on prognosis or treatment. Due to a lack of therapeutic trials in this area, the interest from clinicians wanes and patients are left without further treatment options20. Patients can now be encouraged to adopt the rehabilitation technique known as smell training21, however despite this, patients remain in need of effective additional therapeutic options22 . Patients find the motivation to undertake smell training is harder if no residual olfactory function is left or if they suffer with parosmia, whereby they experience unpleasant sensations when in contact with the stimulus.

PVOL is diagnosed when patients report smell loss that starts with an upper respiratory tract infection and does not recover when the other symptoms disappear; in addition to the presenting timeline, patients lack objective examination findings such as those found in Chronic Rhinosinusitis (CRS) but show reduced or absent smell performance on psychophysical smell testing. Our data published in 2014 and 2019 showed the major impact on quality of life in a survey of 496 patient members of Fifth Sense (registered charity for smell and taste disorders) and in a qualitative research study, in people with olfactory loss of all causes in the UK **23,24**. In specific response to their olfactory impairment, they reported high rates of depression (49%), anxiety (47%), impairment of eating experience (95%), isolation (64%) and relationship difficulties (59%). These findings have been replicated in other studies **25,26** and patients feel that they have not been managed well and that their condition is usually trivialised**24** **27**. Most patients suffer a loss of flavour perception which can adversely affect their appetite, but this can be made even worse if distortions in their sense of smell (such as parosmia) co-exist (67% of PVOL sufferers) **28,29**. They often adopt poor dietary habits with negative impacts on their nutritional status and global health, as they will tend to eat a less varied diet **29-31**. Many sufferers are also concerned about domestic hazards: 72% of the subjects in an online survey of 1000 affected individuals **32** were concerned about the inability to detect a gas leak or a fire and this resulted in dangerous situations for some subjects **33**. Other subjects with reduced olfactory acuity also experienced adverse effects due to exposure to undetected volatile chemicals **32.**

### Current standard of care for PVOL

During a typical episode of PVOL, health service contacts for such a patient will comprise several GP consultations, between one and three outpatient appointments and possibly a prescription of corticosteroids. Assuming the above rates of depression and even if only 10% of anosmia sufferers receive treatment with antidepressants, then we estimate the annual cost could be close to £10 million. Our PPI group discovered that the UK Benefits Agency would consider this problem significant in relation to an application for a disability living allowance. Our prior work has also demonstrated difficulties smell loss patients have in accessing healthcare**34**.

Loss of smell was considered in the *Generate* research prioritisation agenda in 2015**35** but priority was given to CRS as the leading cause of olfactory dysfunction. Guidelines for sinonasal disease exist**36** and research into improving treatment pathways is underway (National Institute of Health Research (NIHR) funded MACRO Programme, Philpott CI)**37**. As outlined above, other causes of smell loss that are not caused by a blockage in the nose, such as PVOL, need more treatments developing to help clinicians provide evidence-based treatments to patients**15**. Although there have been studies exploring the use of various formulations, routes and doses of steroid, no large randomised controlled trials focused on this subset of patients have been conducted. In a randomised controlled trial by Seo et al**38**, 40mg oral prednisolone as monotherapy or combination with 80mg of ginkgo biloba for 4 weeks was shown to have significant improvement in olfactory function. However, this study did not include a control placebo group to ascertain if the improvement was statistically significant in comparison to an untreated group. A recent survey of 120 patients with PVOL showed 48% had received no treatment whatsoever, with 35% receiving nasal steroids and 18% oral steroids; none reported effectiveness of these steroid-based interventions9. The recent Position Paper on Olfactory Dysfunction concluded**15**: “When considering use of systemic corticosteroids, the risk of side effects must be taken into account. At present, evidence-based guidelines regarding the acceptable frequency of systemic corticosteroid use do not exist. It therefore falls to the individual clinician to exercise the appropriate prudence, particularly in cases of non-CRS related olfactory loss, where the evidence supporting steroid use is poor.” Recently a consensus document by the Clinical Olfactory Working Group concluded that oral steroids should be avoided other than to exclude sinonasal inflammatory disease and some favoured using Vitamin A drops**22**.

### Pathophysiology of olfactory dysfunction and rationale for objective assessments

The location of olfactory receptor neurons (ORNs) are unique, in that they are more likely to suffer damage than the protected nerves of the other senses, being exposed to the external environment and subjected to various external agents including pathogens, pollutants and dust. The consequent damage to the olfactory neuroepithelium can lead to neurodegeneration **39** and related reduction in olfactory bulb volume (OBV) **40,41** and right orbital sulcus volume**42**. These changes imply reduced neural activation in amygdala/temporal piriform and insular cortices **43**, brain areas well known to be active during passive perception of odours **44,45**. Although the olfactory system has a unique capacity to regenerate **46**, this process can fail, as occurs following viral injury to the ORNs.

It is the group of viruses that give rise to the common cold which are thought to be the pathological agent in causing the olfactory loss. This includes rhinoviruses (30-50%), parainfluenza (5%), Coronavirus (10-15%), Influenza (5-15%), Coxsackie (<5%), adenoviruses (<5%) and respiratory syncytial viruses (10%), however there are over 200 viruses in total that produce upper respiratory tract infections**47**. The frequency of viruses found in the studies that have examined the aetiological agents vary with parainfluenza virus common in some (e.g. 88%)**48-50**, with others showing differing results and giving prominence to Epstein-Barr virus, rhinovirus, and coronavirus**51**.

The mechanism by which the virus wreaks its havoc usually involves it hijacking the cellular apparatus, but the exact details may depend on the actual virus implicated. Rhinovirus, for instance, causes a selective neutrophil and monocyte recruitment to occur. The inflammatory cascade that ensues includes an increase in bradykinin, cytokine, chemokine and sICAM-1 concentrations**52**.The response in an immunocompetent individual involves T-lymphocyte activation allowing the viral pathogen to be eliminated.

With specific respect to the olfactory apparatus, these viruses appear to cause some partial loss of receptors in the olfactory epithelium. Ultrastructurally, studies have revealed a decrease in the number of olfactory receptor cells and nerve bundles with squamous metaplasia occurring in a few cases**53**. This reduction in the number of ciliated olfactory receptors means at the epithelial surface there is a lack of dendrites and vesicles, therefore, a decrease in the area available for odour molecule detection**54**.

Changes in OBV have been shown to correlate with changes in smell performance on psychophysical testing**55**, thus we have chosen this as a primary outcome measure for this proof of concept study. Significant relationships have also been shown between the volume of key olfactory areas and psychophysical testing including the piriform cortex, orbitofrontal cortex (OFC), and the insular cortex **56**. Prior work by Suebert et al showed a strong connection between right orbitofrontal cortex volume and general olfactory performance **42**. This demonstrated that larger grey matter volume in the orbital sulcus was the key link. Therefore, in summary we have proposed OBV as the primary outcome measure and right orbital sulcus volume as a secondary measure.

### 5.1.1 Explanation for choice of comparators

Vitamin A offers a potential treatment option for olfactory loss due to ORN damage. It is metabolised to retinoic acid (RAc), which as a transcription regulator, is important in tissue development and regeneration57. Embryogenesis and ORN regeneration appear to include RAc signalling 58. When Vitamin A is converted to retinoic acid, it is thought to control olfactory progenitor cell differentiation; therefore it will prevent exhaustion of the stem cell supply or accumulation of non-functional immature neurons (that are not processing odours)59. This regeneration of mature neurons from stem cells in adults is limited to the olfactory neuroepithelium, which produces new ORN and to the subgranular zone, where new granule cells are supplied to the dentate gyrus of the hippocampus in the brain; interneuron production for the olfactory bulbs are also possibly influenced by these stem cells causing an effect in the subventricular zone of the brain60. Therefore, it is theorised that topical Vitamin A treatment will encourage regeneration of the olfactory epithelium which is damaged by respiratory viruses responsible for the common cold and help to restore the sense of smell in sufferers.

A recent systematic review we conducted identified 4 previous studies that included vitamin A as a treatment modality for olfactory loss61. The first, a case series of 56 patients, described some subjective responses to high-dose systemic therapy given by injection, but this was a mixed group of patients with differing aetiologies 62. A subsequent study for 37 patients with liver cirrhosis treated with **oral** vitamin A demonstrated 2-5 fold improvement in performance on threshold tests for 4 odours as a by-product of vitamin A treatment, also given orally 63. More recently, a double-blind placebo-controlled trial using **oral** Vitamin A at the same dose as the previous study of 10,000 IU per day for 3 months was carried out in 52 patients with PVOL and post-traumatic smell loss. However, no obvious treatment effect for oral delivery was shown 64. A review of the trial registries shows that there is currently 1 trial underway in Canada (ClinicalTrials.gov Identifier: NCT03574701) but this includes a mixed group of patient aetiologies including those being treated for CRS and is underpowered to address the group of PVOL patients specifically and does not include any mechanistic evaluation such as brain imaging.

In 2017 a pseudo-randomised clinical trial of Vitamin A conducted in Germany, this time delivered intranasally at a dose of 10,000 IU per day for 8 weeks, showed that in 124 patients with PVOL, a minimum clinically important difference in olfactory function was seen in 37% of those receiving vitamin A compared to 23% receiving smell training alone 65. Key attributes of this study included beneficial response in patients with PVOL, apparent evidence of patient safety and acceptability at the stated dose, duration and delivery mechanism (intranasally) with minimal side-effects evident. Due to unbalanced treatment groups and the pseudo-randomisation, the study lacked scientific rigour and requires further proof of concept evidence for intranasal Vitamin A.

The dosing regimen proposed below has been based on the previous study undertaken by Hummel et al 65 and should reduce the potential for adverse effects. Our systematic review allowed for reflection on other options to be considered in this study: corticosteroids (see above), oral theophylline, oral alpha-lipoic acid and intranasal sodium citrate61. The key advantage of vitamin A intranasally is avoidance of oral administration and systemic side effects. The alternative use of theophylline requires invasive and more costly blood monitoring with potentially more unpleasant side effects. The effect of sodium citrate in our recent trial was transient (effect lasted 30-120 minutes) with 1 in 3 responding in the active treatment arm but with a need for more data on long-term benefits66. The potential benefits of alpha-lipoic acid were based on only 14 patients improving in one small trial67. Peanut oil is the base for the Vitamin A drops and will therefore act as the placebo.

**5.1.2 Rationale for this study**

This study aims to provide scientific rationale that will facilitate a larger randomised controlled trial (RCT). Pending the outcome of this study, we would anticipate moving straight to an RCT proposal as soon as is practically possible following the data analysis. The underlying premise is to provide a low cost but effective treatment option for patients with PVOL in the future which in the wake of the Covid-19 pandemic is needed now more than ever.

## 5.2 Objectives

Overarching aim: To undertake a two-arm randomised controlled trial of intranasally delivered vitamin A versus placebo (peanut oil) to determine proof of concept

Specific objectives of this study are:

1. To assess the impact of Vitamin A on olfactory bulb and right orbital sulcus volume using MRI volumetric data and white matter structural connectivity between these brain areas with diffusion MRI (dMRI).
2. To assess the impact of Vitamin A on neural activation in the amygdala, temporal piriform and insular cortices as indicated by an average signal increase of 0.9 in the primary olfactory cortex68 using fMRI
3. To determine impact of intranasally delivered Vitamin A on psychophysical smell test scores
4. To assess the impact of intranasally delivered Vitamin A on quality of life.

The outcomes used to assess these objectives will help provide physical evidence of a positive effect of vitamin A on the olfactory system.

## 5.3 Trial Design

The study will be conducted as a two-arm randomised placebo-controlled trial comparing 10,000 IU once daily Vitamin A self-administered intranasal drops versus peanut oil drops delivered over 12 weeks in patients with post viral olfactory loss.

MHRA have confirmed study proposed is a mechanistic study and not classified as a CTIMP and therefore does not fall under the need for Clinical Trials Authorisation.

## 6.1 Site Selection

The trial sponsor has overall responsibility for site and investigator selection and has delegated this role to the CI and NCTU.

### 6.1.1 Study Setting

The study will take place at the University of East Anglia Brain Imaging Centre (UBIC) with recruitment of patients from the tertiary referral Norfolk Smell & Taste Clinic located at the James Paget University Hospital.

### 6.1.2 Site/Investigator Eligibility Criteria

The sites for this study have been preselected on the basis of being the location of the brain imaging centre (UBIC) and the Norfolk Smell & Taste Clinic. The following sites will be included:

* University of East Anglia
* James Paget University Hospital

#### 6.1.2.1 Principal Investigator’s (PI) Qualifications and Agreements

The investigator(s) must be willing to sign an investigator statement to comply with the trial protocol (confirming their specific roles and responsibilities relating to the trial, and that their site is willing and able to comply with the requirements of the trial). This includes confirmation of appropriate qualifications, familiarity with the appropriate use of any investigational products, agreement to comply with the principles of GCP, to permit monitoring and audit as necessary at the site, and to maintain documented evidence of all staff at the site who have been delegated significant trial related duties.

#### 6.1.2.2 Resourcing at site

The investigator(s) should be able to demonstrate a potential for recruiting the required number of suitable subjects within the agreed recruitment period (i.e. the investigator(s) regularly treat(s) the target population). They should also have an adequate number of qualified staff and facilities available for the foreseen duration of the trial to enable them to conduct the trial properly and safely.

Sites will be expected to complete a delegation of responsibilities log and provide staff contact details.

The site should have sufficient data management resources to allow prompt data return to NCTU.

## 6.2 Site approval and activation

On receipt of the signed investigator statement, approved delegation of responsibilities log and staff contact details, written confirmation will be sent to the site PI. The trial manager or delegate will notify the PI in writing of the plans for site initiation. Sites will not be permitted to recruit any patients until a letter for activation has been issued. The Trial Manager or delegate will be responsible for issuing this after a green light to recruit process has been confirmed by Sponsor’s legal representative.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor, HRA, and which was given favourable opinion by the Research Ethics Committee (REC). The PI or delegate must document and explain any deviation from the approved protocol and communicate this to the trial team at NCTU. At UEA the SOP process for this must be followed which results in formal notification to sponsor's legal representative.

## 6.3 Participants

### 6.3.1 Eligibility Criteria

#### 6.3.1.1 Participant selection

Adult patients presenting to ENT surgeons with symptoms of anosmia/hyposmia with or without parosmia with no conductive cause identified for their symptoms and a clear history of a preceding upper respiratory tract infection (including Covid-19) that has resolved clinically leaving the olfactory disorder.

There will be **NO EXCEPTIONS** (waivers) to eligibility requirements at the time of randomisation. Questions about eligibility criteria should be addressed PRIOR to attempting to randomise the participant.

The eligibility criteria for this trial have been carefully considered and are the standards used to ensure that only medically appropriate participants are entered. Participants not meeting the criteria should not be entered into the trial for their safety and to ensure that the trial results can be appropriately used to make future treatment decisions for other people with similar diseases or conditions. It is therefore vital that exceptions are not made to these eligibility criteria.

Participants will be considered eligible for enrolment in this trial if they fulfil all the inclusion criteria and none of the exclusion criteria as defined below.

#### 6.3.1.2 Participant Inclusion Criteria

A partial or total loss of smell due to post-viral olfactory loss as confirmed on history, examination and with a smell test (TDI) score of <31/48 and within 3 years of the precipitating viral infection.

#### 6.3.1.3 Participant Exclusion Criteria

1. Participants with a history of:
   1. chronic rhinosinusitis with/without nasal polyposis
   2. severe nasal septal deviation
   3. Major prior head injury
   4. congenital olfactory loss
   5. use of concurrent intranasal medications or possible medications know to affect olfaction
   6. chronic renal disease
   7. chronic hepatic disease
   8. allergy to peanuts, soy or vitamin A (drops contain peanut oil)
2. Significant medical, surgical or psychiatric disease that in the opinion of the PI would affect subject safety or influence the study outcomes
3. Currently taking oral vitamin A supplements, anticoagulants or tetracyclines
4. Age of less than 18
5. Pregnant women and women of a childbearing age not using an effective contraceptive
6. Participants unsuitable for MRI due to metal implants, such as pacemaker etc, as is standard for MRI scanning or who move excessively during scanning.
7. Evidence from endoscopy or the initial MRI scan of:
   1. Participants with any endoscopic findings of:
      1. Chronic rhinosinusitis with/without nasal polyposis
      2. Severe nasal septal deviation (preventing passage of 4mm endoscope)
      3. Other inflammatory sinonasal disease
   2. Participants with MRI changes indicating oedema in the sinuses and/or olfactory clefts
8. Any participant with a combined OBV of >85mm3 will be excluded as it is unlikely they will demonstrate significant increase in overall volume based on previous studies of OBV69.
9. Participation in another trial in the last 4 months.

#### 6.3.1.5 Co-enrolment Guidance

Concurrent participation in clinical trials of investigational medical products is not permitted. However, participants may be entered into other observational studies given prior agreement from the CI of both studies.

#### 6.3.1.6 Screening Procedures and Pre-randomisation Investigations

Written informed consent to enter and be randomised into the trial must be obtained from participants, after explanation of the aims, methods, benefits and potential hazards of the trial and **BEFORE** any trial-specific procedures. The only procedures that may be performed in advance of written informed consent being obtained are those that would be performed on all patients in the same situation as a usual standard of care.

Patients will have attended TNSTC and undergone a nasal endoscopy and the Sniffin’ Sticks olfactory test (to determine their TDI score) as standard of care. They will then attend their baseline visit at the UBIC. See recruitment details below; these arrangements may depend on the influence of the COVID-19 pandemic. If necessary, in order to avoid the usual endoscopic examination, potential participants may be screened by telephone and if they do not have other rhinological symptoms (such as nasal blockage, rhinorrhoea and facial pressure), then they will proceed into the study, with the caveat that if the subsequent MRI scan shows inflammatory sinonasal disease, they will be excluded from the study at that point and replaced with another recruit in order to achieve the planned sample size. We anticipate the chances of finding sinonasal disease after screening will be 3% or less70, and thus may only affect 1 or 2 participants. Women of a childbearing age will be asked to confirm that they have a suitable means of contraception and present a negative pregnancy test result prior to being permitted to enter the trial.

## 6.4 Interventions

Study participants will take Vitamin A drops (Vitadral Aristo Pharma GmbH, Berlin, Germany) self-administered intranasally via a dropper at a dose of 10,000 IU (2 drops per nostril) once daily for 12 weeks. The dose and duration is based on the recent study by Hummel *et al* 65. All participants will receive the drops via post from the James Paget University Hospital pharmacy once notified by the APOLLO research associate. The participants will be taught by the research associate how to apply the drops using the Kaiteki position and a leaflet and a video will also be available for them to look at in their own time via the research team’s website (www.uea.ac.uk/rhinology-group). The Kaiteki position is a way of ensuring the drops roll into the olfactory cleft at the top of the nose where the smell receptors are located71. Both Vitamin A and Peanut oil drops for the placebo arm will be received and administered in the same way.

### 6.4.1 Arm A

#### 6.4.1.1 Products

Vitadral Topfen Oral Drops (Aristo Pharma) Vitadral Aristo Pharma GmbH, Berlin, Germany

#### 6.4.1.2 Treatment Schedule

Daily application for 12 weeks. Self-administered intranasally via a dropper at a dose of 10,000 IU once daily.

#### 6.4.1.3 Dispensing

Dispensed by the hospital pharmacy at James Paget University Hospital

#### 6.4.1.4 Dose Modifications, Interruptions and Discontinuations

The threshold for adherence will be 80% allowing for minor interruptions (of no more than 7 days) of treatment. Participants will also be expected to take the total daily dose of 10,000 IU on at least 80% of occasions.

### 6.4.2 Arm B

#### 6.4.2.1 Products

Matched Placebo (Peanut oil)

#### 6.4.2.2 Treatment Schedule

Daily application for 12 weeks. Self-administered intranasally via a dropper at a dose 2 drops to each nostril once daily.

#### 6.4.2.3 Dispensing

Dispensed by the hospital pharmacy at James Paget University Hospital

#### 6.4.2.4 Dose Modifications, Interruptions and Discontinuations

Equivalent volume to Vitamin A

### 6.4.3 Accountability

Criteria for discontinuing or modifying allocated interventions for a given trial participant:

* Patient experiencing uncomfortable side-effects
* Patient develops a contraindication to MRI scanning e.g. has a pacemaker fitted

### 6.4.4 Compliance and Adherence

Regular contact with participants by the research team will seek to ensure that they remain engaged with the study. A trial website will also provide support videos and feedback for nasal drop use. Weekly phone calls to participants will aim to ensure that a minimum of 80% of the 12-week course of drops is completed and obtain feedback on any local side-effects.

### 6.4.5 Contraindications

* A combination of vitamin A and retinoic acid or its chemical derivatives (e.g. medicinal products for the treatment of skin diseases) should be avoided, as there is a risk of vitamin A overdosing.
* With simultaneous use of high doses of Vitamin A and drugs to inhibit blood coagulation (dicumarol, warfarin), the anticoagulant effect can be increased.
* The simultaneous use of antibiotics such as tetracyclines and vitamin A can lead to an increase in intracranial pressure.
* Neomycin (an antibiotic) and cholestyramine and colestipol (medicines used to lower blood lipids) can inhibit vitamin A absorption in the intestine.
* Due to the already established benefit of olfactory training (OT) in PVOL72, study participants will be asked to refrain from undertaking OT during the study period. Other intranasal treatments will also be discouraged during the study period.

### 6.4.6 Overdose of Trial Medication

Hypervitaminosis is unlikely due to the topical delivery of treatment and was not evident in participants of the previous study. Therapeutic measures in the event of an overdose would be to discontinue the preparation and contact the study team.

### 6.4.7 Protocol Treatment Discontinuation

In consenting to the trial, participants are consenting to trial treatments, trial follow-up and data collection. However, an individual participant may stop treatment early or be stopped early for any of the following reasons:

* Unacceptable treatment toxicity or adverse event
* Inter-current illness that prevents further treatment
* Any change in the participant’s condition that in the clinician’s opinion justifies the discontinuation of treatment
* Withdrawal of consent for treatment by the participant

As participation in the trial is entirely voluntary, the participant may choose to discontinue trial treatment at any time without penalty or loss of benefits to which they would otherwise be entitled. Although not obliged to give a reason for discontinuing their trial treatment, a reasonable effort should be made to establish this reason, whilst remaining fully respectful of the participant’s rights.

Participants who discontinue protocol treatment, for any of the above reasons, should remain in the trial for the purpose of follow up and data analysis.

## 6.5 Outcomes

### 6.5.1 Primary Outcomes

Primary outcome measure: Olfactory bulb volume (OBV) (on MRI scan)

### 6.5.2 Secondary Outcomes

1. Right orbital sulcus volume (MRI scan)
2. Blood-oxygen- level-dependant (BOLD) -signal in primary olfactory **areas; amygdala, piriform, and insula (fMRI scan)**
3. Psychophysical smell test (TDI) score
4. Olfactory disorders questionnaire (ODQ) score

### MRI scan OBV and sulcus measurements and fMRI changes

For participants in both arms of the study, MRI readings will be taken at baseline and at 12 weeks to allow for olfactory bulb measurement and use of dynamic changes on fMRI. Each visit will be conducted in the UEA Brain Imaging Centre (UBIC) at the same time of day each time to avoid issues of circadian rhythm. Individual T1-weighted MRI images will be acquired for volumetric analyses of grey matter changes. T2-weighted BOLD imaging sequences will be acquired for analyses of functional changes in resting state and while participants passively perceive odours (phenethyl alcohol (rose-like pleasant smell) and hydrogen sulphide (rotten eggs -unpleasant) and odourless air in repeated trials. Each stimulus will be delivered through an olfactometer for 3 seconds per trial, followed by a variable delay of 4-10 seconds after which the participant will perform a three-alternative forced-choice response indicating which odour they perceived on an MRI-compatible button box. Participants should expect to take 1.5 hours for the fMRI. Additionally, diffusion tensor imaging (DTI) sequences will be deployed to investigate white matter fibre integrity. The olfactometer enables a supply of warmed odourised air flows with a delivery hose to the nose that contains no ferro-magnetic materials, thus compatible with an MRI scanner (Burghart, Wedel, Germany).

### Psychophysical smell test score and ODQ

All participants will undergo the Sniffin’ Sticks smell test at baseline (for inclusion into the study), and a repeat test will be undertaken at the 3-month visit to assess subjective olfactory performance. The smell test has 3 parts – threshold (T), discrimination (D) and identification (I); this results in a composite TDI score out of 48. The test utilises a set of pens containing odours or blanks (depending on which section of the test) and using the alternative forced choice method, participants are asked to identify the presence of the odour (threshold), the odd odour out of 3 (discrimination) or the correct odour from 4 options (identification), as the pens are waved under their nose. This test takes 20-25 minutes to complete. The olfactory disorders questionnaire takes about 5-10 minutes to complete, assesses quality of life impact and has been previously validated by the CI in a UK setting73.

## 6.6 Participant Timeline

Patients will attend their baseline visit at the UBIC. See recruitment details below; these arrangements may depend on the influence of the COVID-19 pandemic. If necessary, in order to avoid the usual endoscopic examination, potential participants may be screened by telephone and if they do not have other rhinological symptoms (such as nasal blockage, rhinorrhoea and facial pressure), then they will proceed into the study, with the caveat that if the subsequent MRI scan shows inflammatory sinonasal disease, they will be excluded from the study at that point and replaced with another recruit in order to achieve the planned sample size. We anticipate the chances of finding sinonasal disease after screening will be 3% or less70, and thus may only affect 1 or 2 participants.

After completion of the baseline visit (1.5-2 hours), participants will commence their nasal drops as instructed by the research team. They will be invited to reattend after 3 months for repeat assessment. After the second visit their participation in the study will be complete. See flowchart below for further details.

Schedule of enrolment, interventions, and assessments.

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | STUDY PERIOD | | | | | |
|  | Enrolment | Baseline | Allocation | Weekly checks | 3 months | Close-out | |
| TIMEPOINT | *-t1* | 0 | *t1* |  | *t2* | *tx* | |
| ENROLMENT: |  |  |  |  |  |  | |
| Eligibility screen | X |  |  |  |  |  | |
| Informed consent | X |  |  |  |  |  | |
| *Smell test, ODQ* | X |  |  |  |  |  | |
| Allocation |  | X |  |  |  |  | |
| INTERVENTIONS: |  |  | | | | | |
| *Vitamin A drops or Peanut oil drops* |  |  |  |  |  | |  |
|  |  |  |  |  |  | |  |
| ASSESSMENTS: |  | Weekly diaries throughout 3 months | | | | | |
| *Smell test (TDI score)* | X |  | X |  | X | |  |
| *OBV* |  |  | X |  | X | |  |
| *MRI and fMRI measures* |  |  | X |  | X | |  |
| *ODQ* |  |  | X |  | X | |  |

### 6.6.1 Patient Assessments

The APOLLO RA will undertake patient assessments in liaison with the JPUH team. It is anticipated that the JPUH RN will undertake prior screening of patients to avoid unnecessary visits to UBIC by participants who will not be eligible for the study.

### 6.6.3 Early Stopping of Follow-up

If a participant chooses to discontinue their trial treatment, they should continue to be followed up as closely as possible to the follow-up schedule defined in the protocol, providing they are willing. They should be encouraged and facilitated not to leave the whole trial, even though they no longer take the trial treatment. If, however, the participant exercises the view that they no longer wish to be followed up, this view must be respected and the participant withdrawn entirely from the trial. NCTU should be informed of the withdrawal in writing using the appropriate APOLLO trial documentation. Data already collected will be kept and included in analyses according to the intention-to-treat principle for all participants who stop follow up early.

Participants who stop trial follow-up early will be replaced.

### 6.6.4 Participant Transfers

If a participant moves from the area making continued follow up at site inappropriate, then they should be withdrawn from the trial.

### 6.6.5 Loss to Follow-up

Participants who are lost to follow up (despite attempts to contact them on 3 occasions), will be withdrawn from the study.

### 6.6.6 Trial Closure

The end of the trial is defined as 12 months following the last follow-up visit of the last patient randomised, to allow for data entry and data cleaning activities to be completed.

## 6.7 Sample Size

Fifty-seven participants who are eligible and have provided informed consent will be recruited and randomised on a 2:1 ratio, with 38 allocated to receive the intervention and 19 to receive placebo. This sample size will aim to detect a difference of 20 mm3 in OBV with 90% power based on a 2:1 allocation ratio, and based on a standard deviation of 20mm3 69 with an assumed 10% drop-out rate accounting for the above MRI-based exclusions. This will also allow greater capture of the number of adverse events in the intervention arm and ensures that the placebo arm contains 19 patients, an accepted minimum amount required for a feasibility or pilot study.

## 6.8 Recruitment and Retention

### 6.8.1 Recruitment

Recruitment will take place over 12 months. Patients will be identified through the Smell & Taste Clinic at the James Paget University Hospital as well as from members of Fifth Sense who will be alerted via membership channels including social media and mailshots to contact the study team. Those identified from either source will be sent the relevant patient information pack (by mail/e-mail/website download). The CI and RN will screen potential participants to check their eligibility Those who contact the team via Fifth Sense will be asked to seek a GP referral to the Smell & Taste Clinic to enable recruitment. Depending on Covid-19 related restrictions at the time of commencing the study, the initial screening may be undertaken via telephone with the final eligibility checks undertaken at the baseline study visit at the UEA Brain Imaging Centre (UBIC) when they undergo their first MRI scan. In this latter scenario, the baseline Sniffin’ Sticks test will also be performed at this visit by the research associate. All necessary precautions for Covid-19 screening will be undertaken and national and local policies will be adhered to for any face-to-face contact, depending on the measures in place at the time of study commencement. Enrolment and informed consent will be undertaken by the RA at UBIC.

### 6.8.2 Retention

Regular monthly contact with participants by the research team will seek to ensure that they remain engaged with the study. A trial website will also provide support videos and feedback for nasal drop use. Weekly phone calls to participants will aim to ensure that a minimum of 80% of the 12-week course of drops is completed.

## 6.9 Assignment of Intervention

### 6.9.1 Allocation

#### 6.9.1.1 Sequence generation

Participants will be randomly assigned to either the intervention or the placebo group with a 2:1 allocation as per a computer-generated randomisation schedule. The randomisation will be performed after the initial baseline MRI scan to ensure the eligibility criteria are fully met.

#### 6.9.1.2 Allocation concealment mechanism

The random allocation order will be generated before the trial begins and concealed from the research team by a CTU statistician. An interactive web randomisation system will be used by a member of the CTU team who is not blinded to the intervention, for allocation of participants to one of the two groups, after the informed consent and baseline measures have been completed.

#### 6.9.1.3 Allocation Implementation

The patient will be allocated a participant number at time of consent. When the results of the baseline tests, and all other pre-designated questions are completed in the CRF, the allocated staff will then have access to the randomisation process for that participant. The treatment allocation will be revealed and linked to that participant number.

### 6.9.2 Blinding

All participants, care providers and outcome assessors will be blinded to the treatment allocation. Accidental unblinding will be logged and monitored to ensure that appropriate steps are taken to prevent this from reoccurring. The data analysts will not be blinded as they are required to know the group for the preparation of the DMEC reports.

### 6.9.3 Emergency Unblinding

Where possible, requests for emergency unblinding of individuals should be made via NCTU in agreement of the Chief Investigator, will be sought. However, in circumstances where there is insufficient time to make this request or for agreement to be sought, the treating clinician can make the decision to unblind immediately. This can be done via the study database. The Chief Investigator will have special logins which will allow unblinding of individual patients. This is closely audited within the database management system. All instances of unblinding should be recorded and reported to NCTU by the local principal investigator, including the identity of all recipients of the unblinding information.

## 6.10 Data Collection, Management and Analysis

### 6.10.1 Data Collection Methods

Each participant will be given a unique trial Participant IDentification number (PID). Data will be collected at the time-points indicated in the Trial Schedule. The preferred method of data collection is direct online entry of data onto the central database, stored on servers based at NCTU by members of the APOLLO trial team working within each research site. Data may be entered onto paper Case Report Forms (CRFs) prior to entry onto the database. Staff will receive training on data collection and use of the online system.

Data collection, data entry and queries raised by a member of the APOLLO trial team will be conducted in line with the NCTU and trial specific Data Management Standard Operating Procedure. Identification logs, screening logs and enrolment logs will be kept at the trial site in a locked cabinet within a secured room.

Clinical trial team members will receive trial protocol training. All data will be handled in accordance with the Data Protection Act 2018 and the General Data Protection Regulation (GDPR) (EU) [2016/679](https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32016R0679).

Participant identifiable data will be stored on a Participants Database to enable patients to be contacted by site staff for the purpose of contacting participants and sending questionnaires; and the central trial team for the purpose for sending newsletters during the trial. There will be a clear logical separation of participant identifiable data from the trial data.

### 6.10.2 Data Management

Data will be entered under the participants PID number onto the central database stored on the servers based at NCTU. Access to the database will be via unique, individually assigned (i.e. not generic) usernames and passwords, and only accessible to members of the APOLLO trial team at NCTU, and external regulators if requested. The servers are protected by firewalls and are patched and maintained according to best practice. The physical location of the servers is protected physically and environmentally in accordance with University of East Anglia’s General Information Security Policy 3 (GISP3: Physical and environmental security).

The database and associated code will be developed by NCTU Data Management, in conjunction with the APOLLO trial team. The database software provides a number of features to help maintain data quality, including; maintaining an audit trail, allowing custom validations on all data, allowing users to raise data query requests, and search facilities to identify validation failure/ missing data.

After completion of the trial the database will be retained on the servers of NCTU for on-going analysis of secondary outcomes.

The identification, screening and enrolment logs, linking participant identifiable data to the pseudoanonymised PID, will be held locally by the trial site. This will either be held in written form in a locked filing cabinet or electronically in password protected form on hospital computers. After completion of the trial the identification, screening and enrolment logs will be stored securely by the sites for 5 years unless otherwise advised by NCTU.

### 6.10.3 Non-Adherence and Non-Retention

The consent form will explain that if a participant wishes to withdraw from the study the data and samples acquired prior to that point will be retained. Reason for withdrawal will be recorded, if given, as will loss to follow up.

Non-adherence to trial medication will be assessed by weighing the bottles for residual volume when returned to the RA at the 3-month visit; the pre-trial bottle weight will be known to the pharmacy.

Where patients are unable or unwilling to continue to undertake study measurements/activities, the decision as to whether the participant should continue with trial treatment and/or follow-up assessments will be at the discretion of the CI.

### 6.10.4 Statistical Methods

#### 6.10.4.1 Outcomes

The comparison between groups will be based on the intention-to-treat population. The OBV will be compared between the two groups using a two-sample t-test. The brain imaging data will be analysed in SPM12 (UCL, London, UK)74, based on probabilistic maps of region of interests derived from a meta-study75, as well as anatomical markers76,77. In order to address the primary outcome measure; changes in OBV, will be compared to the control group using a two-sample test of outcome measures adjusted for baseline. The second outcome measure of changes in orbitofrontal sulcus depth will also be analysed in the same manner. To understand the wider impact of anosmia on brain function we will use resting state fMRI analysis with novel brain network and machine learning pipelines77. In a similar approach to Lee et al. who investigated olfactory functional networks in Parkinson's disease dementia and Alzheimer's dementia78,79, we will use an regions of interest seed-based approach for the comparison of functional changes in the resting-state networks. The seed regions of interest we aim to investigate are the olfactory bulb, olfactory tract, piriform cortex, and orbitofrontal cortex (OFC) between groups. While OBV measurement has been widely used to investigate olfactory diseases, white matter integrity analyses are more recent. DTI analyses allows the evaluation of different fibre properties in white matter. Güllmar et al. showed that improvement of olfaction in chronic rhinosinusitis patients following surgery correlated with olfactory performance and DTI measures of cortical change80. In our study the diffusion MRI analysis will be conducted with a reproducible, robust and efficient dMRI processing pipeline81. We will also plan to use a diffusion tensor imaging sequence for the olfactory bulb and then use a white-matter skeleton co-registration approach (e.g. tract-based spatial statistics) to reduce inter-individual differences82. The use of advanced machine learning algorithms will allow us to go beyond simple correlations to make predictions and rank the effects of the intervention 82-84.

#### 6.10.4.2 Statistical Analysis Plan

A full SAP will be produced prior to the analysis of any data. The SSC will be given the opportunity to comment on the SAP prior to it being signed-off by the CI and lead statistician.

#### 6.10.4.3 Additional Analyses

An adjusted analysis will also be conducted adjusting for the corresponding baseline measure using a general linear model. A similar analysis to the OBV and sulcus measurement analysis will be used for the TDI score from the Sniffin’ Sticks psychophysical smell test and the ODQ.  If the assumptions of the tests are not met, then non-parametric approaches will be used. We will also assess the correlation between the OBV measurements and the TDI scores using Pearson’s correlation coefficient.

#### 6.10.4.4 Analysis Population

The intention-to-treat population will consist of all participants who were randomised who have outcome data in the group that they were allocated to, regardless of adherence. No multiple imputation will be undertaken for this study.

#### 6.10.4.5 Missing Data

Patterns of missing data will be described, but no imputation will be undertaken in this early phase study.

## 6.11 Data Monitoring

### 6.11.1 Data Monitoring Oversight

The trial will be led by the chief investigator working with the co-applicants who provide expertise in statistics, methodology, data management, functional imaging and computational modelling. A research associate (RA) will be employed to undertake the day-to-day running of the study. Study activities and responsibilities will be documented in an overarching trial management agreement and signed by the James Paget University Hospital, CI and the Clinical Research Network. Randomisation and data management will be facilitated by the use of an in-house randomisation and data management system. The RA will maintain essential documentation with a study Master File and the CI will manage the regulatory and ethics approval process.

The Project Management Group (CI, RA, research nurse, other co-applicants as needed) research team will meet regularly (monthly during set up phase then 2 monthly during the recruitment phase) to draft, review and approve key essential documents including any documentation needed for research governance. The study steering committee will meet twice a year and include all co-applicants and affiliated research team members including our PPI representative. Online meeting platforms will be used where needed. Ad hoc meetings will be added where needed for any issues arising including safety monitoring. The Independent Steering Committee will encompasses the role of a safety committee for the purposes of this trial.

### 6.11.2 Interim Analyses

There are no planned interim efficacy analyses.

### 6.11.3 Data Monitoring for Harm

#### 6.11.3.1 Safety reporting

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial. We will notify the REC and sponsor of any unexpected serious adverse reactions. We will use the sponsors SAE form for this purpose. We will adhere to the Health Research Authority guidelines as follows85:

Table 1: Adverse Event Definitions

|  |  |
| --- | --- |
| **Adverse Event (AE)** | Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and which does not necessarily have a causal relationship with this product. |
| **Adverse Reaction (AR)** | Any untoward and unintended response to an investigational medicinal product related to any dose administered/trial treatment |
| **Unexpected Adverse Reaction (UAR)** | An adverse reaction, the nature or severity of which is not consistent with the applicable product information (eg Investigator’s Brochure for an unauthorised product or summary of product characteristics (SPC) for an authorised product or treatment |
| **Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)** | Any AE or AR that at any dose:   * results in death * is life threatening\* * requires hospitalisation or prolongs existing hospitalisation\*\* * results in persistent or significant disability or incapacity * is a congenital anomaly or birth defect * or is another important medical condition\*\*\* |
| \* the term life threatening here refers to an event in which the patient is at risk of death at the time of the event; it does not refer to an event that might hypothetically cause death if it was more severe (eg a silent myocardial infarction)  \*\* Hospitalisation is defined as an in-patient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisation for pre-existing conditions (including elective procedures that have not worsened) do not constitute an SAE  \*\*\* Medical judgement should be exercised in deciding whether an AE or AR is serious in other situations. Important AEs or ARs that may not be immediately life threatening or result in death or hospitalisation, but may seriously jeopardise the participant by requiring intervention to prevent one of the other outcomes listed in the table (e.g. a secondary malignancy, an allergic bronchospasm requiring intensive emergency treatment, seizures or blood dyscrasias that do not require hospitalisation, or development of drug dependency). | |

Adverse events include:

* an exacerbation of a pre-existing illness
* an increase in the frequency or intensity of a pre-existing episodic event or condition
* a condition (regardless of whether PRESENT prior to the start of the trial) that is DETECTED after trial drug administration. (This does not include pre-existing conditions recorded as such at baseline – as they are not detected after trial drug administration)
* continuous persistent disease or a symptom present at baseline that worsens following administration of the trial treatment

Adverse events do NOT include:

* Medical or surgical procedures: the condition that leads to the procedure is the adverse event
* Pre-existing disease or a condition present before treatment that does not worsen
* Hospitalisation where no untoward or unintended response has occurred eg elective cosmetic surgery
* Overdose of medication without signs or symptoms

The research associate will record any non-serious adverse events to allow for an ongoing comparison between arms for the data monitoring and safety committee in any subsequent RCT.

#### 6.11.3.2 Other Notifiable Adverse Events

None

#### 6.11.3.3 Exempted Adverse Events

None

#### 6.11.3.4 Procedures to follow in the event of female participants becoming pregnant

We will withdraw any female participant who becomes pregnant. Female participants will be asked to inform the RA if they become pregnant during the course of the study.

#### 6.11.3.5 Investigator responsibilities relating to safety reporting

All non-serious AEs and ARs, whether expected or not, should be recorded in the patient’s medical notes and reported in the toxicity (symptoms) section of the Follow-up Form and sent to NCTU within 24 hours of awareness of the event. SAEs and SARs should be notified to NCTU and to the sponsor immediately the investigator becomes aware of the event (in no circumstance should this notification take longer than 24 hours).

##### 6.11.3.5.1 Seriousness assessment

When an AE or AR occurs, the investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in Table 1. If the event is classified as ‘serious’ then an SAE form must be completed and NCTU (or delegated body) notified immediately.

##### 6.11.3.5.2 Severity or grading of Adverse Events

The severity of all AEs and/or ARs (serious and non-serious) in this trial should be graded according to CTCAE grading criteria and be assigned a grade 1-5.

##### 6.11.3.5.3 Causality

The investigator must assess the causality of all serious events or reactions in relation to the trial therapy using the definitions in Table 2.

Table 2: Causality definitions

|  |  |  |
| --- | --- | --- |
| Relationship | Description | Event type |
| Unrelated | There is no evidence of any causal relationship | Unrelated SAE |
| Unlikely to be related | There is little evidence to suggest that there is a causal relationship (eg the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant’s clinical condition or other concomitant treatment) | Unrelated SAE |
| Possibly related | There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant’s clinical condition or other concomitant treatment) | SAR |
| Probably related | There is evidence to suggest a causal relationship and the influence of other factors is unlikely | SAR |
| Definitely related | There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out. | SAR |

If an SAE is considered to be related to trial treatment, and treatment is discontinued, interrupted or the dose modified, refer to the relevant Interventions sections of the protocol.

##### 6.11.3.5.4 Expectedness

If there is at least a possible involvement of the trial intervention (including any comparators), the CI must assess the expectedness of the event. An unexpected adverse reaction/event is one that is not reported in the approved version of the Reference safety Information (RSI) in the Vitadral SmPC, or one that is more frequently reported or more severe than previously reported. See appendix 1 for a list of expected toxicities/reactions associated with the interventions being used in this trial; given topically to the nose, some recipients may experience mucosal irritation on application. If a SAR is assessed as being unexpected it becomes a SUSAR (suspected, unexpected, serious adverse reaction), REC reporting guidelines apply (see Notifications sections of the protocol).

#### 6.11.3.6 Notifications

##### 6.11.3.6.1 Notifications by the Investigator to NCTU

NCTU and the sponsor must be notified of all SAEs within 24 hours of the investigator becoming aware of the event.

Investigators should notify NCTU of any SAEs and other Notifiable Adverse Events (NAEs) occurring from the time of randomisation until 30 days after the last protocol treatment administration. SARs and SUSARs must be notified to NCTU until trial closure. Any subsequent events that may be attributed to treatment should be reported to the MHRA using the yellow card system (https://yellowcard.mhra.gov.uk/the-yellow-card-scheme/).

The SAE form must be completed by the investigator (the consultant named on the delegation of responsibilities list who is responsible for the participant’s care) with attention paid to the grading, causality and expectedness of the event. In the absence of the responsible investigator, the SAE form should be completed and signed by a member of the site trial team and emailed as appropriate within the timeline. The responsible investigator should check the SAE form at the earliest opportunity, make any changes necessary, sign and then email to NCTU. Detailed written reports should be completed as appropriate. Systems will be in place at the site to enable the investigator to check the form for clinical accuracy as soon as possible.

The minimum criteria required for reporting an SAE are the trial number and date of birth, name of reporting investigator and sufficient information on the event to confirm seriousness. Any further information regarding the event that is unavailable at the time of the first report should be sent as soon as it becomes available.

The SAE form must be scanned and sent by email to the trial team at NCTU if applicable.

Participants must be followed up until clinical recovery is complete and laboratory results have returned to normal or baseline values, or until the event has stabilised. Follow-up should continue after completion of protocol treatment and/or trial follow-up if necessary. Follow-up SAE forms (clearly marked as follow-up) should be completed and emailed to NCTU as further information becomes available. Additional information and/or copies of test results etc may be provided separately. The participant must be identified by trial number, date of birth and initials only. The participant’s name should not be used on any correspondence and should be blacked out and replaced with trial identifiers on any test results.

##### 6.11.3.6.2 NCTU responsibilities

Medically qualified staff at NCTU and/or the Chief Investigator (CI or a medically qualified delegate) will review all SAE reports received. In the event of disagreement between the causality assessment given by the local investigator and the CI, both opinions and any justifications will be provided in subsequent reports.

The delegated staff at NCTU will review the assessment of expectedness and complete this section of the report, delegating to unblinded staff where relevant for a blinded trial.

NCTU is responsible for the reporting of SUSARs to the REC and sponsor as appropriate. Fatal and life threatening SUSARs must be reported to the competent authorities within seven days of NCTU becoming aware of the event; other SUSARs must be reported within 15 days.

NCTU will keep investigators informed of any safety issues that arise during the course of the trial.

### 6.11.4 Quality Assurance and Control

#### 6.11.4.1 Risk Assessment

The Quality Assurance (QA) and Quality Control (QC) considerations for the APOLLO trial are based on the standard NCTU Quality Management Policy that includes a formal Risk Assessment, and that acknowledges the risks associated with the conduct of the trial and proposals of how to mitigate them through appropriate QA and QC processes. Risks are defined in terms of their impact on: the rights and safety of participants; project concept including trial design, reliability of results and institutional risk; project management; and other considerations.

QA is defined as all the planned and systematic actions established to ensure the trial is performed and data generated, documented and/or recorded and reported in compliance with the principles of GCP and applicable regulatory requirements. QC is defined as the operational techniques and activities performed within the QA system to verify that the requirements for quality of the trial related activities are fulfilled.

#### 6.11.4.2 Central Monitoring at NCTU

NCTU staff will review Case Report Form (CRF) data for errors and missing key data points. The trial database will also be programmed to generate reports on errors and error rates. Essential trial issues, events and outputs, including defined key data points, will be detailed in the APOLLO trial Data Management Plan.

#### 6.11.4.3 On-site Monitoring

The frequency, type and intensity of routine and triggered on-site monitoring will be detailed in the APOLLO Quality Management and Monitoring Plan (QMMP). The QMMP will also detail the procedures for review and sign-off of monitoring reports. In the event of a request for a trial site inspection by any regulatory authority, NCTU must be notified as soon as possible.

##### 6.11.4.3.1 Direct access to participant records

Participating investigators must agree to allow trial related monitoring, including audits, REC review and regulatory inspections, by providing access to source data and other trial related documentation as required. Participant consent for this must be obtained as part of the informed consent process for the trial.

#### 6.11.4.4 Trial Oversight

Trial oversight is intended to preserve the integrity of the trial by independently verifying a variety of processes and prompting corrective action where necessary. The processes reviewed relate to participant enrolment, consent, eligibility, and allocation to trial groups; adherence to trial interventions and policies to protect participants, including reporting of harms; completeness, accuracy and timeliness of data collection; and will verify adherence to applicable policies detailed in the Compliance section of the protocol. Independent trial oversight complies with the NCTU trial oversight policy.

In multi-centre trials this oversight is considered and described both overall and for each recruiting centre by exploring the trial dataset or performing site visits as described in the APOLLO Quality Management and Monitoring Plan.

##### 6.11.4.4.1 Study Team

A research associate (RA) will be employed to undertake the day-to-day running of the study. A Senior Trial Manager (STM) at Norwich CTU will assist the CI and RA with study planning, study set-up prior to appointment of the RA, oversight of study management and adherence to CTU practices. The STM provides expertise in trial design, methodology and conduct throughout the project. A Senior Statistician at Norwich CTU will provide advice throughout the study and statistical oversight and will be supported by a project statistician throughout the study.

##### 6.11.4.4.2 Study Management Group

A Study Management Group (SMG) will be set up to assist with developing the design, co-ordination and strategic management of the trial. The SMG includes the members of the Study Team as well as other co-applicants who provide expertise in statistics, methodology, data management, functional imaging and computational modelling. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the SMG terms of reference.

The SMG will meet regularly (monthly during set up phase then 2 monthly during the recruitment phase) to draft, review and approve key essential documents including any documentation needed for research governance.

##### 6.11.4.4.3 Independent Study Steering Committee

The Independent Study Steering Committee (SSC) will include two experienced physicians independent of the trial and is the independent group responsible for oversight of the study in order to safeguard the interests of participants. The SSC provides advice to the CI, NCTU, the funder and sponsor on all aspects of the trial through its independent Chair. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the SSC terms of reference. Due to the nature of the trial proposed, the SSC will incorporate the role of a safety committee as defined below:

• To detect any trends, such as increases in un/expected events, and take appropriate action

• To seek additional advice or information from investigators where required

• To evaluate the risk of the trial continuing and take appropriate action where necessary

##### 6.11.4.4.5 Trial Sponsor

The University of East Anglia is the trial sponsor. The role of the sponsor is to take on responsibility for securing the arrangements to initiate, manage and finance the trial. The Sponsor is responsible for ensuring that the study meets the relevant standards and makes sure that arrangements are put and kept in place for management, monitoring and reporting. The University of East Anglia has delegated some Sponsor’s activities to the CI and NCTU, these are documented in the collaboration agreement and delegation of responsibilities.

# 7 Ethics and Dissemination

## 7.1 Research Ethics and Health Research Authority Approval

Before initiation of the trial at any clinical site, the protocol, all informed consent forms and any material to be given to the prospective participant will be submitted to the relevant REC and to HRA for approval. Any subsequent amendments to these documents will be submitted for further approval.

The rights of the participant to refuse to participate in the trial without giving a reason must be respected. After the participant has entered the trial, the clinician remains free to give alternative treatment to that specified in the protocol, at any stage, if s/he feels it to be in the best interest of the participant. The reasons for doing so must be recorded. After randomisation the participant must remain within the trial for the purpose of follow up and data analysis according to the treatment option to which they have been allocated. However, the participant remains free to change their mind at any time about the protocol treatment and follow-up without giving a reason and without prejudicing their further treatment.

## 7.2 Competent Authority Approvals

The APOLLO trial is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is not required in the UK.

## 7.3 Other Approvals

Documentation will need to be submitted to the R&D Department at each NHS Site in order to gain confirmation of capacity and capability prior to the study being initiated at that site. Confirmation from the site will take the form of a site agreement signed by both the Sponsor and the relevant site.

A copy of the local R&D approval and of the Participant Information Sheet (PIS) and consent form on local headed paper must be forwarded to the co-ordinating centre before participants are randomised to the trial.

The protocol has received formal approval and methodological, statistical, clinical and operational input from the NCTU Protocol Review Committee.

## 7.4 Amendments

Amendments to the Protocol and other documents (e.g. changes to eligibility criteria, outcomes, sample size calculations, analyses) will be agreed by the TMG. Such amendments will be forwarded to the Sponsor for confirmation as to whether it is either substantial or non-substantial and will then be submitted to the Health Research Authority or Ethics Committee for categorisation and approval. Once the amendment has been categorised it will be sent to relevant sites for consideration in accordance with standard HRA processes and timescales. Amendments must not be implemented until HRA approval is received and sites have either confirmed acceptance or, no objection has been received within the defined timescale. Notification will be sent by NCTU to trial personnel to confirm when an amendment can be implemented.

## 7.5 Consent or Assent

Patients will be provided with a Patient Information Sheet (PIS) and given time to read it fully. Following a discussion with a medical qualified investigator or suitable trained and authorised delegate, any questions will be satisfactorily answered and if the participant is willing to participate, written informed consent will be obtained. During the consent process it will be made completely and unambiguously clear that the participant is free to refuse to participate in all or any aspect of the trial, at any time and for any reason, without incurring any penalty or affecting their treatment.

Consent will be re-sought if new information becomes available that affects the participant’s consent in any way. This will be documented in a revision to the patient information sheet and the participant will be asked to sign an updated consent form. These will be approved by the ethics committee prior to their use.

A copy of the approved consent form is available from the NCTU trial team.

## 7.6 Confidentiality

Any paper copies of personal trial data will be kept at the participating site in a secure location with restricted access. Confidentiality of patient’s personal data is ensured by not collecting patient names on CRFs and limiting access to personal information held on the database at NCTU. At trial enrolment the patient will be issued a participant identificationnumber and this will be the primary identifier for the patient, with secondary identifiers of month and year of birth and initials.

The patient's consent form will carry their name and signature. These will be kept at the trial site, with a copy sent to NCTU for monitoring purposes. This copy will be destroyed once checks are complete. Consent forms will not be kept with any additional patient data.

## 7.7 Declaration of Interests

The investigators named on the protocol have no financial or other competing interests that impact on their responsibilities towards the scientific value or potential publishing activities associated with the trial.

## 7.8 Indemnity

The NHS indemnity scheme will apply to the potential liability of the Sponsor for harm to participants arising from the conduct of the research. Insurance and indemnity to meet the potential legal liability of the sponsor for harm to participants arising from the design and management of the research will be provided by UEA.

## 7.9 Finance

APOLLO is fully funded by an NIHR RfPB grant number 201978. It is not expected that any further external funding will be sought.

## 7.10 Archiving

The investigators agree to archive and/or arrange for secure storage of APOLLO trial materials and records for 5 years after the close of the trial unless otherwise advised by the NCTU

## 7.11 Access to Data

Requests for access to trial data will be considered, and approved in writing where appropriate, after formal application to the TMG. Considerations for approving access are documented in the TMG Terms of Reference. The final trial dataset will be held at Norwich CTU on a secure server and will be accessible by the research team. UEA will own the foreground IP (as employer of the Chief Investigator) and JPUH will have rights via a royalty free licence to use for their own non-commercial purposes and patient benefit. Any exploitation shall be managed through the University’s Intellectual Property Team who shall put into place appropriate royalty share agreements although it is not anticipated that this will be the case as further research will be required.

## 7.12 Ancillary and Post-trial Care

There are no plans to offer trial treatment to individuals participating in this study after its conclusion. Vitamin A supplementation is available without prescription should the participant wish to continue with this following the study. Any participants needing additional care will be returned to the Norfolk Smell & Taste clinic for management. This will also apply to those who have persistent olfactory dysfunction at the end of the study.

## 7.13 Publication Policy

### 7.13.1 Trial Results

Beyond informal and specialist publications, we also plan to use wider communication channels and in conjunction with PPI input and we will utilise existing websites such as Fifth Sense ([www.fifthsense.org.uk](http://www.fifthsense.org.uk)) to engage the public.

### Projected outputs:

We expect the outputs of this study to enable a subsequent randomised controlled trial of Vitamin A versus placebo with an internal pilot to establish the place of a suitable placebo. As mentioned at the top of the application. The PPI input will ensure that our outputs are also publicly available in a way that is meaningful and relevant to patients. As aforementioned in the application, we had previously submitted a study proposal to the NIHR EME funding stream to conduct a randomised controlled trial but were asked to provide more proof of concept. We have already prepared a draft RCT proposal in partnership with the Norwich Clinical Trials Unit and would plan to develop this further in light of the findings of this proof-of-concept study.

#### Expected research outputs:

* The protocol will be published in Trials open access journal
* Open access publications in appropriate journals such as Rhinology and Chemical Senses
* Conference presentations and proceedings
* Funding proposal for a randomised controlled trial

Authorship for journal publications will be in line with the ICJME guidelines <http://www.icmje.org> .

The results of the trial will be disseminated regardless of the direction of effect.

### 7.13.2 Authorship

Ownership of the data arising from the study resides with the trial team. The publication policy will be in line with rules of the International Committee of Medical Journal Editors. The TMG will decide on authorship.

### 7.13.3 Reproducible Research

The trial will be registered on the International Standard Randomised Clinical Trials Number (ISRCTN) website granting public access to the trial outcomes. In addition, the clinical study protocol will be submitted for publication. Every effort will be made to grant access to the participant level dataset subject to TMG approval.

# 8 Protocol Amendments

|  |  |  |
| --- | --- | --- |
| Protocol Version | Date | Summary of Changes |
|  |  |  |

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# 10 Appendices

## Appendix 1: SmPC for Vitamin A

1. Name of the medicinal product  
   Vitadral® drops 30.2 mg / ml, drops for oral use, solution
2. Qualitative and quantitative composition  
   Active substance: Retinol palmitate 1ml (about 27 drops) solution Vitadral drops Contains 30.2 mg of retinol palmitate (equivalent 54900 I.E.). Other ingredients with known effect: Butylhydroxytoluene, peanut oil  
   Full list of excipients see section 6.1.  
   Note: Retinol palmitate is one of the substances that collectively referred to as "vitamin A" (see paragraph 5. "Pharmacological Properties").
3. Pharmaceutical form clear, yellow drops for oral use, solution
4. Clinical information  
   1. Applications  
      Therapy of a vitamin A deficiency, that cannot be compensated nutritionally.
   2. Posology and method of administration dosage - See table  
      For other intake quantities are preparations with higher active ingredient content available. Vitadral drops can be dropped on a spoon taken undiluted together with unheated drinks or foods. The duration of the treatment depends on the course of the disease.
   3. Contraindications  
      Vitadral drops should not be used in  
      - Hypersensitivity to retinol palmitate (Vitamin A), peanut or soy or one of those ingredients mentioned in section 6.1.  
      - therapy with retinoic acid and its derivatives;  
      - intracranial pressure increase;  
      - hypervitaminosis A;  
      - incompatibility of an ingredient of Vitadral drops
   4. Warnings and precautions for the application

Vitamin A substitution may occur in hemodialyzed Patients with hypervitaminosis A in combination with hypercalcemia. The vitamin A status of such patients should be therefore monitored. Patients with a severe form of Hypertriglyceridemia type V exhibit an increased risk for a hypervitaminosis A. The vitamin A status of such patients should therefore be monitored.

In patients with alcohol abuse, co-administration of alcohol and vitamin A increases hepatotoxicity. The supplementation should therefore be done with great restraint.  
Butylated hydroxytoluene may locally cause skin irritation (eg, contact dermatitis), irritation of the eyes and mucous membranes.

Other notes:

Liver, milk and butter are the main sources of vitamin A (retinol and retinyl ester). In addition, it occurs in egg yolk, cream, cheese and fish, but not in plant foods. In contrast, provitamin A (carotene and carotenoids) u. a. included in carrots, spinach, tomatoes and Brussels sprouts. Liver may contain vitamin A in concentrations that are especially prone to childhood malformations in early pregnancy can lead (up to over 100,000I.E./100 g). Pregnant women in the first trimester and women who want to have children should therefore abstain from consuming liver and instead should increasingly consume carotene-rich juices and vegetables. In the second and third trimesters, the consumption of liver is harmless with respect to the vitamin A concentrations contained therein. Women of child-bearing potential should generally not consume large quantities of liver at once. More frequent small portions (50 - 75 g) are preferable.

Vitamin A deficiency states are extremely rare in healthy people in geographic regions with high quality and adequate food supply. However, a marked vitamin A deficiency requiring treatment can occur during maldigestion and malabsorption in the context of gastrointestinal diseases, such as gastrointestinal diseases. Crohn's and Sprue's, in ileojejunal bypass, pancreatic diseases, parenteral nutrition over a prolonged period and as a result of alcohol abuse. Vitamin A deficiency diets are characterized by characteristic ophthalmological symptoms, according to WHO  
classified according to their severity:

XN = night blindness  
X1A = conjunctival xerosis  
X1B = bitot spots  
X2 = corneal xerosis.

X3A = corneal ulceration or keratomalacia (less than one third of the corneal surface)  
X3B = keratomalacia (greater than one third of the corneal surface)  
XS = corneal scarring  
XF = fundus xerophthalmia

* 1. Interaction with other medicinal products and other interactions

High doses of vitamin A may increase the anticoagulant effect of dicumarol and warfarin. Tetracyclines, in combination with vitamin A, can increase the intracranial pressure. A combination of vitamin A and retinoic acid or retinoic acid derivatives should be avoided because of the risk of hypervitaminosis. Co-administration of vitamin A with colestyramine, colestipol or neomycin may result in decreased absorption of vitamin A.

* 1. Fertility, pregnancy and lactation

Vitadral drops should not be used because of the risk of childhood malformations  
- in pregnancy in daily doses above 10,000 I.E  
- in women of childbearing age without reliable conception protection, if the possibility of pregnancy exists in daily doses above 10,000 I.U.

However, if vitamin A supplementation is required, the recommended maximum daily dose is 2.4 mg Retinol Equivalents or 8,000 I.U. and the maximum single dose 0.9 mg retinol equivalents or 3,000 I.U. Vitamin A is placental and passes into breast milk. When taking high doses of vitamin A during pregnancy, there is a risk of childhood malformations.

* 1. Effects on ability to drive and ability to drive or operating machines

No studies on the effects on the ability to drive and use machines have been performed.

* 1. Undesirable effects

The frequency of adverse reactions is based on the following categories:  
Very common (≥1 / 10)  
Common (≥1 / 100 - <1/10)

Occasionally (≥1 / 1,000 - <1/100)

Rare (≥1 / 10,000 - <1 / 1,000)

Very rare (<1 / 10,000)

Not known (frequency can not be estimated from the available data)

Immune system disorders

Rare: Peanut oil rarely causes severe allergic reactions. (See also 4.6 "Fertility, pregnancy and lactation", 4.4 "Special warnings and precautions for use" and 4.9 "Overdose").

Report of suspected adverse reactions

The reporting of suspected adverse reactions after approval is of great importance. It allows continuous monitoring of the benefit-risk balance of the drug. Healthcare professionals are required to report any suspected adverse reactions to the Federal Institute for Drugs and Medical Devices,

Department of Pharmacovigilance, Kurt-Georg-Kiesinger-Allee 3, D-53175 Bonn, Website: [www.bfarm.de](http://www.bfarm.de).

* 1. Overdose
     1. Symptoms of overdose

At a serum retinol level of more than 1 mg / l, hypervitaminosis A can be assumed. In pregnant women, a teratogenic effect is possible. Acute hypervitaminosis A may occur from a single intake of about 500 mg retinol equivalents or 1.5 million I.E. Vitamin A in adults, 100 mg or 300,000 I.U. in children and 30 mg or 100,000 I.U. in toddlers.

Symptoms of intoxication include headache, severe fatigue, nausea, papilledema, nocturia, irritability, anorexia and weight loss, mild fever, subcutaneous swelling and tinnitus.

After 24 hours, a massive scaling of the skin occurs. In children, a protrusion of the fontanelle may occur. There is increased fibrinolysis time, decreased Quick value, increased GOT and GPT values.

The symptoms return after 36 hours. Very rarely, anemia and thrombocytopenia occur.

Chronic hypervitaminosis A may be associated with prolonged daily vitamin A administration of 30 mg retinol equivalents or 100,000 I.U. in adults. Vitamin A occurs in children at daily doses of 18,000 to 60,000 I.U. If liver or kidney function is impaired, hypervitaminosis A is possible even at far lower doses. Early symptoms of chronic poisoning include dry and flaky skin, pruritus, rhagades, hair growth disorder, fatigue, bone pain and hemorrhage. Late symptoms include hepatosplenomegaly, hypertrophy of fat-storing liver cells, liver fibrosis, sclerosis of the hepatic central veins, cirrhosis of the liver due to portal hypertension and ascites, pseudotumor cerebri due to pressure increase of the cerebrospinal fluid increase in alkaline phosphatase and serum calcium.

In children, in the course of a chronic intoxication it may lead to premature epiphyseal closure, thickening of the cortical regions, the long bones and in consequence to growth delays.

* + 1. Therapy measures in case of overdose discontinuation of the drug and symptomatic treatment (possibly induced vomiting, gastric lavage or saline laxatives).

1. Pharmacological properties
   1. Pharmacodynamic properties  
      Pharmacotherapeutic group: digestive tract  
      and metabolism, vitamins, vitamin  
      A + D including their combinations,  
      Vitamin A, Monopreparat, Retinol  
      ATC code: A11CA01

Vitamin A is one of the fat-soluble essential vitamins. The term includes retinol and its esters, d. H. all substances that have the biological effect of retinol because they are converted into retinal and retinoic acid. Provitamins are beta-carotene and about 50 carotenoids, which have at least one unsubstituted ring in them  
beta configuration.

The indication of the biological vitamin A effect is given in international units (I.E.) and in retinol equivalents (RE). According to the WHO, one retinol equivalent (RE) corresponds to 1 μg of retinol, 6 μg of beta-carotene and 12 μg of a carotenoid which acts as a pro-vitamin. An International Unit Vitamin A corresponds to 0.3 μg retinol, 0.34 μg retinol acetate and 0.55 μg retinol palmitate equivalents, respectively.

Vitamin A influences the protein synthesis and the synthesis of glycolipids and glycoproteins in the target cells, containing mannose and galactose. It regulates cell growth and differentiation through altered gene expression of enzymes and growth factors required for cell development and cell regeneration. Essentially, three areas of action can be distinguished:

- Retinol acts on growth and differentiation of the epithelial and mesenchymal structures of the bone.  
- Vitamin A affects reproductive and embryonic development (spermatogenesis, oogenesis, placental development and embryonic morphogenesis).  
- Retinal affects the visual process. A marked deficiency of vitamin A leads to xerophthalmia in the anterior segment of the eye (cornification and dehydration of the connective and cornea, which can even lead to blindness). Listening, tasting and smelling are also dependent on sufficient vitamin A supply.

* 1. Pharmacokinetic properties

Following oral administration, retinol and its esters are almost completely absorbed in the presence of bile acids. As retinyl esters, they are added by chylomicrons and transported to the liver and stored there. This results in a high retinyl ester plasma level immediately after vitamin A intake. Retinol is released from the liver and transported to the target organs by retinol-binding proteins (RBP). Retinol or its metabolites active in the target organ are bound there to cytosolic receptor proteins. The average concentration of retinol in the liver is 1 to 3 mg / l, in plasma 0.3 to 0.7 mg / l. Vitamin E increases the tissue storage of vitamin A. The half-life of retinyl esters in the liver is 50 to 100 days. The half-life decreases with heavy alcohol consumption.

Even taking into account the concentration of retinol binding protein (RBP) plasma levels do not allow a reliable diagnosis of hypovitaminosis A because of the high liver storage and the not yet fully understood, peripheral regulation of the  
Vitamin A levels. According to the recommendation of the German Nutrition Society, a retinol plasma level of less than 300 ng / l is considered to indicate a vitamin A deficiency. Vitamin A is hydroxylated by cytochrome P-450, then glucuronidated and renally eliminated. Vitamin A is placental and passes into breast milk.

* 1. Preclinical safety data  
     1. Acute toxicity - see paragraph 4.9 Overdose
     2. Chronic toxicity / subchronic toxicity - see paragraph 4.9 Overdose
     3. Mutagenic and tumorigenic potential - There are no indications of mutagenic and carcinogenic effects.
     4. Reproductive toxicity - In animal experiments both vitamin A and vitamin A overdoses are teratogenic.

1. Pharmaceutical information
   1. List of excipients: Butylhydroxytoluene (Ph. Eur.), Peanut oil
   2. Incompatibilities - Not known yet.
   3. Shelf life - The duration of the shelf life is 2 years. Shelf life after opening the container: 8 weeks
   4. Special precautions:

For storage Store in a refrigerator (2 ° C - 8 ° C). If Vitadral drops are cloudy or solidified in the refrigerator when temperatures are too low, they should be reliquefied by gentle warming to room temperature prior to ingestion.  
During use Vitadral drops should be stored at room temperature. Please note the durability after opening the container.

* 1. Nature and contents of container

Brown glass bottles with drip tray and screw cap  
Dropper bottle to 30ml N 1 solution  
Dropper bottle to 50ml N 2 solution  
Unsaleable pattern  
Dropper bottle to 10 × 50ml solution (clinic pack)  
Dropper bottle to 10 × 30ml solution (clinic pack)  
Not all pack sizes may be marketed.

* 1. Special precautions for disposal and other instructions for use:

Any unused product or waste material should be disposed of in accordance with local requirements. When dripping, the bottle must be held vertically.

1. Holder of the authorization  
   Aristo Pharma GmbH  
   Wallenroder street 8-10  
   13435 Berlin  
   Telephone: +49 30 71094-4200  
   Fax: +49 30 71094-4250
2. Approval number  
   3000071.00.00
3. Date of approval /  
   Extension of the approval  
   10.04.2000 / 27.11.2007
4. Status of the information  
   05/2016
5. Sales demarcation  
   only available on prescription

# 11 Principal Investigator compliance statement

Principal Investigator agreement to confirm adherence to the protocol, the UK Policy Framework for Health and Social Care Research and GCP.

APOLLO

**A P**roof-of-Concept Study for Vitamin A Nasal Drops in Post-Viral **Ol**factory **Lo**ss

I, Carl Philpott, confirm:

1. that James Paget University Hospital and University of East Anglia sites are willing and able to comply with the requirements of the APOLLO trial;
2. that I regularly treat the target population and believe the site has the potential for recruiting the required number of suitable subjects within the agreed recruitment period (figures included in the trial recruitment plan);
3. that I have sufficient time to properly conduct and complete the trial within the agreed trial period;
4. that I have supplied an up to date curriculum vitae, GCP certificate and/or other relevant documentation requested by NCTU, to demonstrate that I am qualified by education, training and experience to assume responsibility for the proper conduct of the trial at this study site;
5. that I am thoroughly familiar with the appropriate use of the investigational products as described in the protocol, in the current Investigator Brochure (if applicable), in the product information and in other information sources provided by NCTU;
6. that I have an adequate number of qualified staff and adequate facilities available for the foreseen duration of the trial to conduct the trial properly and safely;
7. that I will maintain a signature and delegation log of appropriately qualified persons to whom I have delegated trial related duties which includes confirmation that each member of staff is appropriately trained (including GCP) for the roles allocated to them, and will ensure this is made available to NCTU in a timely manner on request;
8. a research CV for each member of staff on the delegation log will be stored in the site file according to site policy;
9. that I take responsibility for ensuring all staff delegated trial related duties are adequately informed about the protocol, the investigational product and their trial related duties and functions, and that I will continue to take responsibility for regularly updating them as new information becomes available;
10. that the James Paget University Hospital and University of East Anglia sites have sufficient resources to manage data generated by the trial to allow prompt and complete data and query return to NCTU;
11. that I am aware of, and will comply with, the principles of GCP as given in the APOLLO protocol compliance statement and the applicable regulatory requirements, and that a record of my GCP training is accessible and described on my current curriculum vitae;
12. that a record of GCP training is accessible for all staff delegated responsibilities in relation to the APOLLO trial and who are named and approved on the site signature and delegation of responsibilities log and that individual training evidence will be saved in the site file, for all staff, according to trust policies;
13. that I will permit routine and for-cause monitoring and auditing by NCTU, and inspection by the appropriate regulatory authorities, including the provision of direct access to source data and other participant notes and files as required; and
14. that I agree to archive and/or arrange for secure storage of APOLLO trial materials and records for a minimum of 5 years after the close of the trial unless otherwise advised by the NCTU.

Agreement: Principal Investigator

|  |  |
| --- | --- |
| Name | Carl Philpott |
| Signature | [insert wet signature] |
| Date | [insert date] |

(Please return a copy of this signed agreement (only pages 44 and 45 to the APOLLO to NCTU at [*############@nctu.uea.ac.uk*](http://mailto:#######).)