FUNDED BY NIHR | National Institute for Health Research

THE EFFICACY AND MECHANISMS OF ACTION OF N-3 POLY-UNSATURATED FATTY ACID SUPPLEMENTATION IN PEOPLE WITH NON-STEROIDAL EXACERBATED AIRWAYS DISEASE AND UNCONTROLLED ASTHMA.

Poly-unsaturated fats for improving nasal polyps and asthma (PUFFIN)

Version	1.5
Date	24/08/2023
Sponsor	Norfolk and Norwich University Hospitals NHS Foundation Trust
Trial registration	
CTA #	
NRES #	

Authorisation: Chief Investigator

Name	Professor Andrew Wilson
Role	Chief Investigator
Signature	DocuSigned by: Andrew Wilson E4A13449D8A14DE...
Date	01 September 2023

Authorisation: Sponsor

Name	Julie Dawson
Role	Sponsor Representative
Signature	DocuSigned by: Julie Dawson 4CBAB366CF354A2...

Date	11 September 2023

Authorisation: CTU Director or Representative

Name	Matthew Hammond
Role	Norwich CTU Deputy Director
Signature	DocuSigned by: Matthew Hammond 7153BBAFE90F42E...
Date	04 September 2023

Authorisation: Trial Statistician

Name	Dr Allan Clark
Role	Medical Statistician
Signature	DocuSigned by: Allan Clark AC8ACE6AEBA34CA...
Date	01 September 2023

Table of Contents

1 Administrative information

This document was constructed using the Norwich Clinical Trials Unit (NCTU) Protocol template Version 4. It describes the PUFFIN trial, sponsored by the Norfolk and Norwich University Hospitals NHS Foundation Trust 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 trials(1) The SPIRIT Statement Explanation and Elaboration document(2) 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 Human Tissue (Quality and Safety for Human Application) Regulations 2007, the UK Data Protection Act 2018 and General Data Protection Regulation (GDPR) 2018, and the UK Policy Framework for Health and Social Care Research, the European Directive 2001/20/EC (where applicable) and other national and local applicable regulations. 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

Norfolk and Norwich University Hospitals NHS Foundation Trust is the trial sponsor and has delegated responsibility for the overall management of the PUFFIN trial to the Chief Investigator (CI) and NCTU. Queries relating to sponsorship of this trial should be addressed to the CI or via the trial team.

1.3 Structured trial summary

Primary Registry and Trial Identifying Number	
Date of Registration in Primary Registry	
Secondary Identifying Numbers	173-12-20
Source of Monetary or Material Support	National Institute for Health and Care Research Efficacy and Mechanism Evaluation (EME) Project: NIHR129910
Sponsor	Norfolk and Norwich University Hospitals NHS Foundation Trust
Contact for Public Queries	puffin@uea.ac.uk
Contact for Scientific Queries	Professor Andrew M Wilson Professor of Respiratory Medicine Floor 2, Bob Champion Research and Education Building Rosalind Franklin Road University of East Anglia Norwich Research Park Norwich, Norfolk NR4 7TJ A.M.Wilson@uea.ac.uk
Short Title or Acronym	Poly-unsaturated fats for Improving nasal polyps and asthma (PUFFIN)
Scientific Title	The efficacy and mechanisms of action of n-3 poly-unsaturated fatty acid supplementation in people with non-steroidal exacerbated airways disease and uncontrolled asthma.
Countries of Recruitment	United Kingdom
Health Condition(s) or Problem(s) Studied	Non-steroidal exacerbated airways disease
Intervention(s)	Patients will be randomised on a 1:1 basis to receive either of the following for 24 weeks; ACTIVE ARM:

	6g of Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) in a 1.3:1 ratio; respectively, as six Omega-3 ethyl ester 90 capsules, taken once daily, or in divided doses, with food. or CONTROL ARM: Matched capsules (six) containing palm olein IV 56 taken once daily, or in divided doses, with food.
Key Inclusion and Exclusion Criteria	**Inclusion Criteria:** 1. Aged ≥18 years. 2. Diagnosis of N-ERD (non-steroidal anti-inflammatory drug exacerbated respiratory disease) according to one of the following: i. Positive aspirin challenge plus a history of nasal polyposis or asthma. ii. More than one typical reaction to NSAIDs or aspirin, plus a history of nasal polyposis or asthma. iii. A single typical reaction to NSAIDs or aspirin and a history of nasal polyposis, plus moderate to severe asthma.* iv. History of nasal polyposis plus asthma, plus blood eosinophilia (≥300x10^6/L) or raised FeNO (>25ppb) within the last 12 months, plus urinary leukotriene E4/creatinine >800pg/mg. 3. Asthma control questionnaire (ACQ) mean score of more than 1.5, as this indicates poor control. This is required to ensure there is a clinical need or a requirement to alter medication. 4. Stable disease, as evidenced by a lack of change in asthma therapy within the last 6 weeks. 5. Adequate understanding of English (or Welsh) as participants will be required to complete questionnaires as part of the study. **Exclusion Criteria:** 1. Tolerant to aspirin or NSAID with no respiratory or nasal reaction on exposure. 2. Significant cardiac disease, respiratory disease or other causing respiratory symptoms more than asthma. 3. Severe or uncontrolled co-morbid disease (other than nasal polyps) which is likely to affect the outcome of the study.

	4. Having had an upper or lower respiratory tract infection requiring antibiotics within four weeks of randomisation. 5. Receiving biological agents. 6. Receiving n-3 fatty acid oral supplements or more than two dietary portions of oily fish per week. 7. Current smoker or more than 15 pack-year smoking history. 8. Consumption of more than 21 units of alcohol per week, as alcohol-induced respiratory symptoms are more common in N-ERD. 9. Pregnant or breastfeeding women and those less than 4 weeks postpartum. 10. Women of Child Bearing Potential (WOCBP) not using a highly effective form of contraceptive. 11. Participation in the active phase of another CTIMP or within 4 weeks of last study drug administration. 12. Patients unable to give written informed consent. 13. Hypersensitivity to the active substance, soya, peanut or any of the excipients. *Defined as asthma at least "additional-add on therapy" as defined by the BTS/SIGN158 British guidelines on the management of asthma (https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma).
Study Type	This study is an interventional clinical trial of an investigational medicinal product: a phase III, randomised, placebo-controlled, two arm parallel group, double-blind, multicentre clinical trial. Randomisation will be generated by a secure web-based system on a 1:1 basis with stratification for recruiting site and inclusion into sputum subgroup.
Date of First Enrolment	May 2023
Target Sample Size	98 participants (49 per group); 52 (26 per group) of whom will be recruited into the sputum sub-study.
Primary Outcome(s)	The change in ACQ-6 (asthma control questionnaire) mean score at 24 weeks post-randomisation of PUFA (poly-unsaturated fatty acid) versus placebo.
Key Secondary Outcomes	The following secondary outcomes will be assessed at the timepoints specified: **At 12 weeks and 24 weeks post-randomisation:** • ACQ-7.

	• Mini Asthma Quality of Life Questionnaire. • Health related quality of life (HR-QoL) measured using the EQ5D-5L questionnaire (quality adjusted life years will be estimated). • Sinonasal outcomes test (SNOT). • Forced expiratory volume in 1 second (FEV_1). • Forced expiratory flow at 50% of vital capacity (FEF_{50}). • Fraction of Exhaled nitric oxide (FeNO). • Urinary leukotriene E4 (LTE4) and prostaglandin D2 (PGD2). • Membrane fatty acid composition as assessed by Red Blood Cell (RBC) fatty acid concentration and omega-3 index. **Every 6 weeks over the course of the 24 week period:** • Peak expiratory flow (PEF) and ACQ-6. • Peak nasal inspiratory flow (PNIF) (where possible).

1.4 Roles and responsibilities

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

1.4.1 Protocol contributors

Name	Affiliation	Role
Professor Andrew Wilson	UEA	Chief Investigator
Professor Philip Calder	Southampton University	Co-applicant
Dr Allan Clark	UEA	Senior Medical Statistician
Ms Olivia Fulton	The University of Edinburgh	Co-applicant/lay member
Matthew Hammond	NCTU	Deputy Director of NCTU
Professor Peter Howarth	University of Southampton	Co-applicant
Dr Shuaib Nasser	Addenbrooke's Hospital	Co-applicant

Professor Anne-Marie Minihane	UEA	Co-applicant
Martin Pond	NCTU	Head of Data Management
Dr Glenis Scadding	Royal National Ear, Nose and Throat Hospital	Co-applicant
TBC	UEA	Director of NCTU

1.4.2 Role of trial sponsor and funders

Name	Affiliation	Role
Julie Dawson	NNUH	Sponsor: Research Services Manager
Michael Sheridan	NNUH	Sponsor: Research Grants Coordinator
Jasmine Nabarro	NNUH	Sponsor: Research Study Officer
Kirsty Lloyd-West	NIHR EME	Funder: Research Manager

1.4.3 Trial Team

Name	Affiliation	Role and responsibilities
Professor Andrew Wilson	UEA	Chief Investigator
Dr Allan Clark	UEA	Senior Medical Statistician
Dr Claire West	NCTU	Senior Data Programmer
Matthew Hammond	NCTU	Deputy Director of NCTU
Colin McAlister	NCTU	Trial Manager
Martin Pond	NCTU	Head of Data Management
Sue Stirling	UEA	Statistician
Dr Matthew Felgate	NCTU	Trial Assistant
Juliet High	NCTU	Research Lead

1.4.4 Trial Management Group

Name	Affiliation	Role and responsibilities
Professor Andrew Wilson	UEA	Chief Investigator

Professor Philip Calder	Southampton University	Co-applicant
Dr Allan Clark	UEA	Senior Medical Statistician
Dr Claire West	NCTU	Senior Data Programmer
Dr Tracy Jackson and Emma Kinley	The University of Edinburgh	Patient and Public Involvement Research Fellows
Matthew Hammond	NCTU	Deputy Director of NCTU
Dr Ramesh Kurukulaaratchy	University of Southampton	Principal Investigator
Dr Shuaib Nasser	Addenbrooke's Hospital	Co-applicant
Professor Anne-Marie Minihane	UEA	Co-applicant
Martin Pond	NCTU	Head of Data Management
Dr Glenis Scadding	Royal National Throat, Nose and Ear Hospital	Co-applicant
Sue Stirling	UEA	Statistician
Juliet High	NCTU	Research Lead
Colin McAlister	NCTU	Trial Manager
Professor Ann Marie Swart	UEA	Director of NCTU

1.4.5 Trial Steering Committee

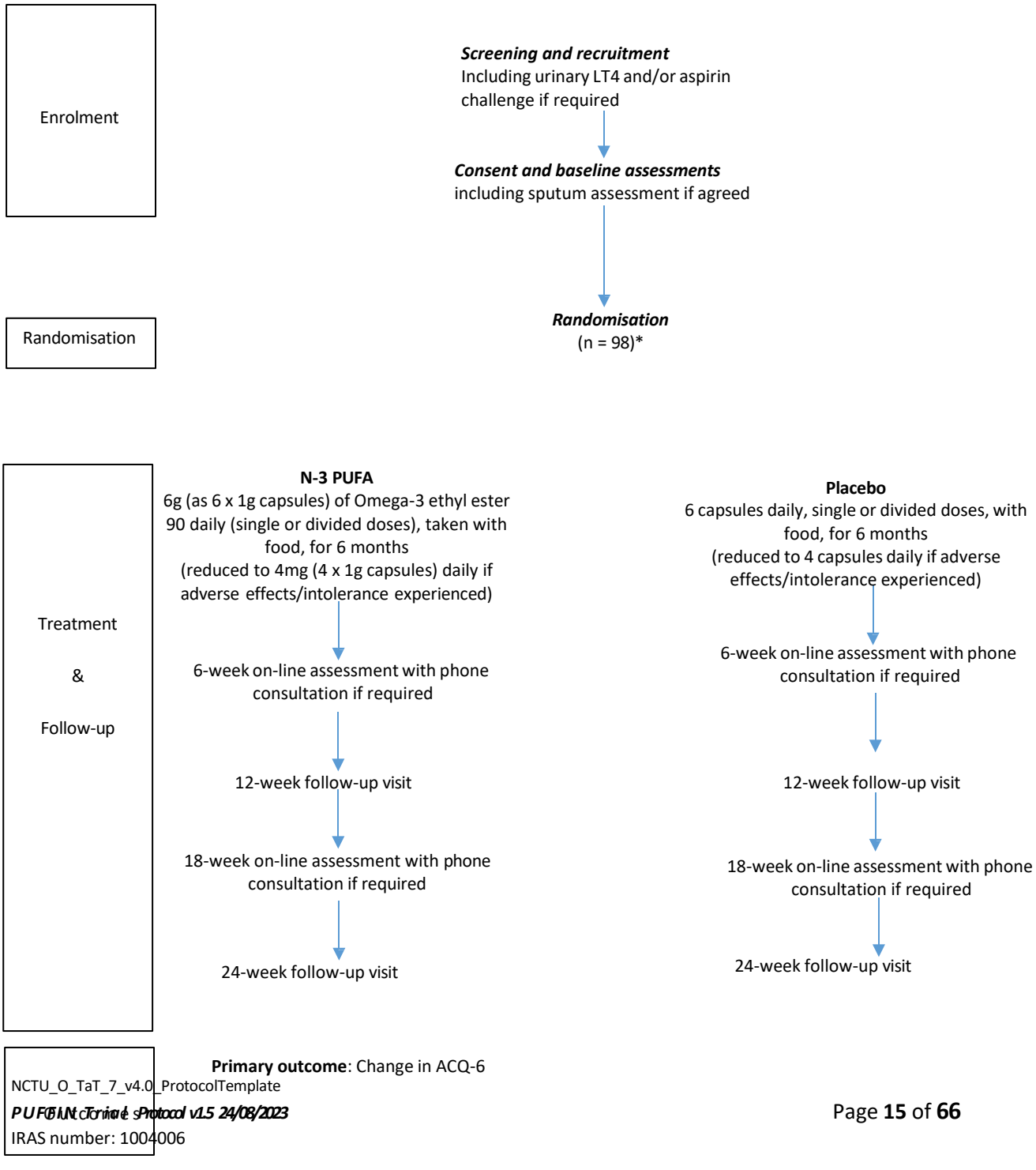
Name	**Affiliation**	**Role and responsibilities**
Professor Adrian Martineau	Queen Mary, University of London	Independent Chair
Dr Thomas Hamborg	Queen Mary, University of London	Independent Statistician
Dr Paul Pfeffer	St Bartholomew's Hospital, Barts Health NHS Trust	Independent Member
TBC	N/A	Patient and Public Involvement Representative
Ms Sandra Kerridge	N/A	Patient and Public Involvement Representative

1.4.6 Data Monitoring Committee

Name	Affiliation	Role and responsibilities
Professor Mark Hull	University of Leeds	Independent Chair
Professor Michael Dewey	King's College London	Independent Statistician
Dr Tom Fardon	Ninewells Hospital Dundee	Independent Member

2 Trial Diagram

Figure 1: PUFFIN trial diagram



Secondary outcomes: PEF, ACQ-7, AQLQ, SNOT-22, FeNO, FEV_1, FEF_{50}, PNIF, blood eosinophils, food frequency questionnaire, RBC fatty acid, sputum eosinophils, sputum SPM, asthma attacks

*52 patients (26 per group for sputum (induced or spontaneous) sub-study
Full eligibility criteria details listed in section 6.3.1, outcomes described in section 6.5 and participant assessments outlined in section 6.6.1.

3 Abbreviations

AA	Arachidonic acid
ACQ	Asthma Control Questionnaire
AE	Adverse Event
AQLQ	asthma quality of life questionnaire
AR	Adverse Reaction
BTS	British Thoracic Society
CI	Chief Investigator
COVID-19	Coronavirus disease 2019
CRF	Case Report Form
CTA	Clinical Trial Authorisation
CTCAE	Common Terminology Criteria for Adverse Events
DHA	Docosahexaenoic Acid
DMC	Data Management Committee (aka IDMC)
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
EPA	Eicosapentaenoic Acid
EQ-5D-5L	Euroqol 5 dimension, 5 level
ERS	European Respiratory Society
EU	European Union
FBC	Full Blood Count
FDA	Food and Drug Administration
FEF_{50}	Forced expiratory flow at 50% of vital capacity
FeNO	Fractional Exhaled Nitric Oxide
FEV	Forced Expiratory Volume
FFQ	Food Frequency Questionnaire
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
HR-QoL	Health related quality of life
ICH	International Conference on Harmonisation
IDMC	Independent Data Monitoring Committee (aka DMC)
IMP	Investigational Medicinal Product
INR	International normalised ratio
ISF	Investigator Site File
ISRCTN	International Standard Randomised Clinical Trial Number
ITT	Intention to Treat
LFTs	Liver Function Tests
LTRA	Leukotriene Receptor Antagonists
MDT	Multi-Disciplinary Team

MHRA	Medicines and Healthcare products Regulatory Agency
NCTU	Norwich Clinical Trials Unit
N-ERD	NSAID Exacerbated Respiratory Disease
NHS	National Health Service
NICE	National Institute for health and Care Excellence
NIHR	National Institute for Health and Care Research
NNUH	Norfolk and Norwich University Hospitals NHS Foundation Trust
N-SAID	Non-steroidal Anti-Inflammatory Drug
PEF	Peak expiratory flow
PGD_2	Prostaglandin D2
PI	Principal Investigator
PID	Participant Identification Number
PIS	Participant Information Sheet
PNIF	Peak nasal inspiratory flow
PUFA	Poly-Unsaturated Fatty Acids
QA	Quality Assurance
QC	Quality Control
QMMP	Quality Management and Monitoring Plan
QoL	Quality of Life
R&D	Research and Development
RBC	Red Blood cell Count
RCT	Randomised Controlled Trial
REC	Research Ethics Committee
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SNOT	Sino-nasal outcomes test
SPC	Summary of Product Characteristics
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File
TMG	Trial Management Group
TMT	Trial Management Team
ToR	Terms of Reference
TSC	Trial Steering Committee
U&Es	Urea and Electrolytes
UAR	Unexpected Adverse Reaction
UEA	University of East Anglia
ULN	Upper Limit of Normal
uLTE4	Urinary leukotriene E4

4 Glossary

None.

5 Introduction

5.1 Background and Rationale

Asthma: a word-wide problem with considerable unmet need.

Asthma is a chronic respiratory problem that can lead to debilitating respiratory symptoms and reduced quality of life. Indeed, it is one of the commonest long-term conditions in the world affecting over 350 million people globally(3). In the UK, which has one of the highest prevalence rates in Europe, there are 5.4 million people with asthma, and asthma results in 93,000 hospitalisations per year(4). The NHS cost of treating asthma in the UK is £1 billion per annum. Indirect costs are at least as high, with significant costs for absenteeism from work and short-term disability(5).

Although there is no cure for asthma, it is generally considered that asthma is a controllable condition; the current expectation is that, with adequate treatment, people with asthma should be free from symptoms and have no risk of asthma attacks. However, a quarter of all asthma patients have poor asthma control(6). Poorly controlled asthma results in greater healthcare and societal costs(7). It has been estimated that the cost of treating people with uncontrolled asthma is $130 per year more than those with controlled asthma(8).

Phenotypes of asthma

It has recently become fully recognised that asthma is a heterogeneous condition(9) with different aetiologies requiring different treatments. Asthma is now considered a syndrome with different phenotypes i.e. including atopic asthma or obesity related non-eosinophilic asthma, which were initially identified by cluster analysis of patient characteristics and response to treatment. This step-change in thinking permits an opportunity to identify and manage different phenotypes of this disease so that targeted medical and other therapies can be provided as part of patient centred care.

One asthma phenotype is non-steroidal anti-inflammatory drug (NSAID) exacerbated respiratory disease (N-ERD)(10). Patients with N-ERD are intolerant to medications that inhibit cyclooxygenase-1 (COX) (NSAID and aspirin) and suffer from asthma, rhinosinusitis and nasal polyps(11). It has previously been referred to as aspirin exacerbated airways disease (AERD), aspirin intolerant asthma, aspirin sensitive asthma or Samter's Triad.

Non-steroidal exacerbation respiratory disease

The prevalence of N-ERD is reported to be between 2% and 25% of people with asthma, depending on severity(12), or 7% (95% CI 5.14 to 9.53) of asthmatics overall(13). It is twice as common in severe or difficult to control asthma. It is often mis-diagnosed, as a diagnosis requires careful history taking regarding symptoms in relation to NSAID/aspirin ingestion and in up to 15% of people the diagnosis is only evident when challenge testing is undertaken(14).

N-ERD is characterised by abnormal arachidonic acid (AA) metabolism, from altered COX and lipoxygenase (ALOX) enzyme activity, resulting in a high concentration of leukotrienes and other pro-inflammatory mediators in the airways(12) causing bronchoconstriction and asthma symptoms. 5-lypoxygenase (ALOX5) is upregulated and along with increased activity of leukotriene synthesis enzymes results in increased leukotriene E4 (LTE4) concentration in tissue and urine(15) whereas reduced COX activity results in reduced prostaglandin E2, an anti-inflammatory mediator in the respiratory system(16). These enzyme activities and their effects on the mediator concentration are enhanced significantly on exposure to COX inhibition by aspirin or NSAIDs. Typically, N- ERD involves upper and lower airway eosinophilia, often with raised blood eosinophil count, and T-helper lymphocyte (TH2) mediated inflammation, probably driven by leukotriene activity. One third of people

have atopy, although there is often no clear association between symptoms and allergen exposure suggesting there may be an innate immune response.

Current treatment for N-ERD

There is currently a lack of clinical guidance regarding N-ERD. People with N-ERD respond poorly to conventional treatment (11, 13, 17), and those with blood eosinophilia often require treatment with anti-IL5 biologics. Neither LTRA nor anti-IL5 biologics alter the pathophysiology of the disease; LTRA block the activity but not the production of leukotrienes. In the UK, aspirin desensitisation is not recommended by guidelines, is poorly tolerated, and uncontrolled studies report slowing of polyp recurrence with lesser benefit on asthma symptoms (11). A limitation of this therapeutic approach is that patients undertaking aspirin desensitisation are at risk of upper gastroenterological symptoms and bleeding, both related to reduced prostaglandin activity, while on maintenance desensitisation therapy.

n-3 PUFA and N-ERD: mechanistic evidence

Manipulation of the AA pathway by dietary supplementation with the n-3 long chain polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), is a potential therapeutic option for people with N-ERD(18). Increasing dietary intake of n-3 PUFA relative to the n-6 fatty acids, which are the main polyunsaturated fat constituent of a western diet, results in an increased EPA to AA ratio in membranes of respiratory and inflammatory cells. As both AA and EPA are metabolised by the same enzymatic pathways (COX, ALOX and cytochrome P450) the production of lipid mediators (eicosanoids and oxylipins) depends on the relative substrate availability. Metabolism of EPA by ALOX5 results in production of 5-series leukotrienes and lipoxins whereas metabolism of arachidonic acid results in production of 4-series leukotrienes and lipoxins. 4-series leukotrienes are more inflammatory than 5-series leukotrienes (19) and are responsible for driving eosinophilic inflammation in asthma and in particular N-ERD(11). In addition, EPA and DHA produce specialised pro-resolving mediators (SPMs) which are potent inflammation resolving molecules(20).

n-3 PUFA and N-ERD: clinical evidence

Systematic reviews of n-3 PUFAs have shown clinical efficacy in rheumatoid arthritis (3-6g/day), anti-inflammatory effects in diabetes (1-6g/day)(21), and anti-allergy effects in children when given to their mothers perinatally (3-4g/day)(22).

In a dose response study, Broughton and colleagues(23) identified responders and non-responders to n-3 PUFA (fitting with the hypothesis that asthma is a syndrome with different phenotypes) in terms of bronchial challenge test findings. The uLTE4 was higher at baseline in responders than in non-responders, increased in both groups with low dose treatment but reduced below baseline with high dose n-3 PUFAs.

A narrative review of n-3 PUFA in asthma summarised the results of nine interventional studies in asthma(24) – none specifically in N-ERD. Overall, only three studies showed null results, with one being a 12-patient study (likely underpowered) and another investigating people with allergic asthma who were symptomatic during the pollen season (likely to have excluded people with N-ERD).

In a recent small dietary manipulation study of 10 people with N-ERD, to increase the n-3 PUFA above 3g/day there was a significant increase in plasma n-3 fatty acids with a corresponding significant reduction (0.17ng/mg creatinine) in uLTE4. Participants had a significant improvement in nasal symptoms (as assessed by the sinonasal outcome test (SNOT-22)) and asthma symptom (assessed by the 7-item ACQ-7)(25).

5.1.1 Explanation for choice of comparators

N-3 PUFA will be compared with matched placebo as this is the most robust way to determine the efficacy and adverse effects of n-3 PUFA. All patients involved in the trial will continue to receive usual care (defined in section 6.4.5).

Treatment allocation is double-blind to reduce potential bias in reporting of the primary outcome and patient reported secondary outcomes.

5.2 Objectives

The primary research hypothesis is that participants treated with n-3 PUFA will have greater improvement in asthma control as measured by the ACQ-6 test at 24 weeks post-randomisation versus participants treated with placebo.

The secondary research hypotheses are that participants treated with n-3 PUFA will:

- Have better asthma control as measured by the ACQ-7 test.
- Have better asthma related quality of life as measured by the asthma quality of life questionnaire (AQLQ).
- Have better health related quality of life measured by the euro-qol (5L-EQ-5D).
- Have better airway calibre as measured by peak expiratory flow (PEF), forced expiratory volume in 1 second (FEV_1) and forced expiratory flow at 50% of vital capacity (FEF_{50}).
- Have better peak nasal inspiratory flow (PNIF).
- Show n-3 PUFA is well tolerated with an acceptable safety profile.

Mechanistic research hypotheses are that the participants treated with n-3 PUFA will:

- Have reduced type 2 airway inflammation as assessed by FeNO, blood eosinophils and sputum eosinophils (subgroup).
- Have reduced urinary leukotriene E4 (uLTE4) and urinary prostaglandin D2 (PGD_2) concentration.
- Have improved Red Blood Cell (RBC) fatty acid concentration.
- Have increased sputum SPMs (subgroup).

Exploratory research hypotheses are:

- that participants treated with n-3 PUFA will have a reduced frequency of asthma attacks.
- those with higher baseline uLTE4 will have a greater response to n-3 PUFA.
- the greatest benefits will be evident in those with low baseline RBC omega-3 index.
- changes in RBC fatty acid concentration will relate to the clinical and mechanistic responses.

5.3 Trial Design

The study is a Phase III double blind, parallel group, 1:1 randomised, placebo controlled, multi-centre, clinical superiority trial of oral n-3 PUFA versus placebo in 98 participants with N-ERD and uncontrolled asthma. Outcomes will be assessed during a treatment period of 24 weeks. The primary endpoint is change in ACQ-6 mean score measured between baseline and 24 weeks post-randomisation of n-3

PUFA versus placebo. A subgroup of 52 participants (26 per group) will be recruited into the sputum sub-study. Randomisation will be performed centrally according to a computer-generated randomisation code by secure automated e-mail from NCTU to site pharmacies only. Stratification factors are: recruiting site and inclusion into sputum subgroup.

5.3.1 Pilot Phase

This study includes an internal pilot phase during which recruitment will be closely monitored by all relevant parties and oversight committees to ensure recruitment of the planned sample size is feasible, safe and ethical.

Progression will be assessed constantly throughout the study according to a traffic light system, with green flagging if the study is progressing above target, red flagging if the study is not likely to deliver and amber flagging if in between. Observed total recruitment will be assessed against expected total recruitment to assign flags. A red flag is indicated where the observed recruitment total is less than 50% of the expected recruitment total for the relative study timepoint.

Actions to be taken according to the progression flagging status;

- Green flagging – study and recruitment to continue as planned.
- Amber flagging – results in increased recruitment of sites and discussion and monitoring of existing sites regarding recruitment and best practice approached.
- Red flagging – three consecutive red flag months will trigger discussion with the Data Monitoring Committee (DMC), Trial Steering Committee (TSC) and Sponsor regarding feasibility of continuing recruitment.

The pilot phase will commence from the date the first recruiting site is activated to recruitment. At the end of the pilot phase a decision will be made by the funder, in consultation with the TSC and DMC, regarding progression to completion of the trial as intended. This will be based on evidence of red flagging with no suggestion of remedial measures. Recruitment will continue until this decision has been reached. All participants recruited during the pilot phase will be included in the further study analyses.

There are no planned interim efficacy analyses.

6 Methods

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 be conducted primarily in secondary and tertiary care hospitals within the United Kingdom.

6.1.2 Site/Investigator Eligibility Criteria

Once a site has been assessed as being suitable to participate in the trial, the trial team will provide them with a copy of this protocol and the Summary of Product Characteristics (SPC).

To participate in the PUFFIN trial, investigators and trial sites must fulfil a set of criteria, agreed by the PUFFIN Trial Management Group (TMG), as defined below. In exceptional circumstances, a site may be excluded from some aspects of the study if they do not meet the criteria below following approval from the CI.

Eligibility criteria:

- A named clinician is willing and appropriate to take Principal Investigator (PI) responsibility.
- Suitably trained staff are available to recruit participants, undertake/observe study assessments and measurements, enter data and collect and store samples.
- The site has a suitable potential patient population to adequately recruit participants from.

Sites are required to have access to a pharmacy able to store, prepare and dispense Investigational Medicinal Product (IMP).

In addition, selected sites will be invited to participate in the PUFFIN sputum sub-study and will approach and consent eligible patients accordingly.

Trial sites meeting eligibility criteria will be issued with a PUFFIN Investigator Site File (ISF) and a pack of documentation needed by the Research and Development (R&D) Department of their Trust to enable the Trust to provide confirmation of capacity and capability to undertake the study.

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 role and responsibilities relating to the trial, and that their site is willing and able to comply with the requirements of the trial (see section 12)). 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. GCP training is required for all staff responsible for trial activities. The frequency of repeat training may be dictated by the requirements of their employing institution or 2 yearly where the institution has no policy, and more frequently when there have been updates to the legal or regulatory requirements for the conduct of clinical trials.

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

6.2 Site approval and activation

The Clinical Trial Authorisation (CTA) for the trial requires that the Medicines and Healthcare products Regulatory Agency (MHRA) is supplied with the names and addresses of all participating site Principal Investigators (PIs). Trial staff at NCTU will perform this task.

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 completed.

The site must conduct the trial in compliance with the protocol as agreed by the Sponsor, Health Research Authority (HRA) and, by the regulatory authority (as appropriate), 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.

A list of activated sites may be obtained from the Trial Manager.

6.3 Participants

6.3.1 Eligibility Criteria

6.3.1.1 Participant selection

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.

In the event a patient fails screening due to presentation with self-reported respiratory tract infection within four weeks of screening or change in asthma medication within six, they may be re-screened once four weeks have elapsed since respiratory tract infection symptom onset or six weeks after change in medication. Patients may be re-screened for any reason if deemed appropriate by the principal investigator.

6.3.1.2 Participant Inclusion Criteria

1. aged ≥18 years. N-ERD does not occur at birth and it rarely occurs in children.
2. diagnosis of N-ERD according to one of the following:
   i. Positive aspirin challenge plus a history of nasal polyposis or asthma.
   ii. More than one typical reaction to NSAIDs or aspirin plus a history of nasal polyposis or asthma.
   iii. A single typical reaction to NSAIDs or aspirin and a history of nasal polyposis plus moderate to severe asthma.*

iv. History of nasal polyposis plus asthma plus blood eosinophilia (≥300x10^6/L) or raised FeNO (>25ppb) within last 12 months plus urinary leukotriene E4/creatinine >800pg/mg.

3. ACQ mean score of more than 1.5 as this indicates poor control. This is required to ensure there is a clinical need or a requirement to alter medication.
4. stable disease, as evidenced by a lack of change in asthma therapy within the last 6 weeks.
5. Adequate understanding of English (or Welsh) as participants will be required to complete questionnaires as part of the study.

* Defined as asthma at least "additional-add on therapy" as defined by the BTS/SIGN158 British guidelines on the management of asthma (https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma).

6.3.1.3 Participant Exclusion Criteria

1. Tolerant to aspirin or NSAID with no respiratory or nasal reaction on exposure.
2. Significant cardiac disease, respiratory disease or other cause for breathlessness which, according to the principal investigator, contributes to the patient's symptoms of breathlessness or other respiratory symptoms to a greater degree than the patient's asthma.
3. Severe or uncontrolled co-morbid disease (other than nasal polyps) which is likely to affect the outcome of the study.
4. Having had an upper or lower respiratory tract infection requiring antibiotics within four weeks of randomisation.
5. Receiving biologic agents.
6. Receiving n-3 fatty acid oral supplements or more than two dietary portions of oily fish per week.
7. Current smoker or more than 15 pack-year smoking history.
8. Consumption of more than 21 units of alcohol per week as alcohol-induced respiratory symptoms are more common in N-ERD.
9. Pregnant or breastfeeding women and those less than 4 weeks postpartum.
10. Women of Child Bearing Potential (WOCBP) not using a highly effective form of contraceptive for the duration of participation in the study. A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy at least 6 weeks prior to enrolment. Pregnancy tests will be required at trial start and at 12 weeks. Highly effective forms of contraception defined as: Methods of birth control which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly such, as implants, injectables, combined oral contraceptives, some intrauterine devices (IUDs), sexual abstinence (defined as refraining from heterosexual intercourse during the entire period of risk associated with the study treatments) or vasectomized partner. This will apply to all women under 55 years of age unless they are postmenopausal or sterile. Postmenopausal is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
11. Participation in the active phase of another CTIMP or within 4 weeks (or the half-life of the drug if longer) of last study drug administration. Participation in observatory trials can occur if agreed between the PI of each trial and where this does not impact the patient or the outcomes of either trial.
12. Patients unable to give written informed consent.
13. Hypersensitivity to the active substance, soya, peanut, or any of the excipients.

Patients will be recruited into the study independent of gender, sexual orientation, marital status, disability, ethnicity, religion or belief. Pregnant women cannot be entered into the study as pregnancy itself may influence the ACQ and therefore the results of the study; however, women >4weeks postpartum can be randomised if they meet the remaining entry criteria. The research will be conducted within secondary care research sites.

Investigators are encouraged to contact the PUFFIN CI, via the NCTU trial team, for guidance in assessing eligibility in relation to exclusion criteria prior to approaching the patient about the trial if required.

6.3.1.4 Co-enrolment Guidance

Concurrent participation in clinical trials of investigational medical products is not permitted. Concurrent participation is defined as within 4 weeks of the last study drug administration (or at least one half-life if longer than 4 weeks). However, participants may be entered into other observational studies given prior agreement from the CI of both studies.

6.3.1.5 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 part of usual standard of care.

6.4 Interventions

6.4.1 Active treatment arm

6.4.1.1 Products

Eicosapentaenoic acid (EPA) plus docosahexaenoic acid (DHA) in a 1.3:1 ratio; respectively, in a white opaque capsule

6.4.1.2 Treatment Schedule

Participants will be asked to take six grams (as 6 x 1g capsules) once daily or in divided doses, with food.

6.4.1.3 Dispensing

Participants will have investigational medicinal product (IMP) dispensed three-monthly. The IMP will be supplied in bottles containing 120 capsules. Where a participant attends a clinic for baseline visit and randomisation is able to take place, the site should aim to dispense the IMP on the same day so that it can be collected by the participant. If a baseline visit has been conducted remotely or due to other exceptional circumstances, the site will have the option to request a courier to send IMP to the participant, see section 6.4.3 below. A dosing card will be dispensed with the IMP stating the required treatment schedule.

6.4.1.4 Dose Modifications, Interruptions and Discontinuations

If a participant forgets to take a dose and it is more than 24 hours after their scheduled dose, they should omit the missed dose and take the next dose when it is due. Participants must not take a double dose to make up for a forgotten dose. Interruptions should be avoided where possible, any reported should be recorded on the eCRF. Participants unable to continue with the IMP due to adverse reaction

or intolerability may reduce to four grams (as 4 x 1g capsules) per day. They must discuss this decision with the site research team and the date of reduction needs to be recorded in the eCRF.

6.4.2 Placebo Arm

6.4.2.1 Products

Placebo 6g palm olein IV 56 in a white opaque capsule (manufactured to appear identical to IMP).

6.4.2.2 Treatment Schedule

Participants will be asked to take six capsules once daily or in divided doses, with food.

6.4.2.3 Dispensing

The PUFFIN trial is a double-blind study and so dispensing for the placebo arm will be managed identically to the active arm (see section 6.4.1.3).

6.4.2.4 Dose Modifications, Interruptions and Discontinuations

If a participant forgets to take a dose and it is more than 24 hours after their scheduled dose, they should omit the missed dose and take the next dose when it is due. Participants must not take a double dose to make up for a forgotten dose. Interruptions should be avoided where possible, any reported should be recorded on the eCRF. Participants unable to continue with the IMP due to adverse reaction or intolerability may reduce to 4 capsules per day. They must discuss this decision with the site research team and the date of reduction needs to be recorded in the eCRF.

6.4.3 Accountability

The UK manufacturer will be responsible for drug accountability of centrally held drug and placebo. Each site pharmacy will be responsible for drug accountability of drug and placebo received at their site, this includes records of drug and placebo received at the pharmacy, dispensed to participants and unused drug and will ensure batch recall is possible in event of it being necessary. The UK manufacturer are also responsible for ensuring centrally held IMP is handled and stored appropriately, and for shipping IMP to each site. Each site pharmacy will be responsible for ensuring IMP and placebo is dispensed accurately to each participant at an appropriate clinic visit or in exceptional circumstances, liaising with NCTU and receiving CI approval, and suitably packaging for couriering to the participant's home address during trial participation (upon receipt of an appropriately signed prescription).

Unused IMP and placebo held centrally will be destroyed by the UK manufacturer. Undispensed IMP and placebo at site pharmacies will be disposed of by the site pharmacy. Participants will be asked to bring unused IMP and packaging to the relevant study visit to assess adherence to trial medication. After final accountability/capsule counts have been undertaken these should be disposed of at site by the research nurse using a special medicine bin (blue lid) or via pharmacies, according to their usual processes and the destruction log updated. Where this is not possible participants will be encouraged to dispose of the capsules via community pharmacies.

6.4.4 Compliance and Adherence

Compliance to study treatment, in the form of returned capsule counts, will be monitored as part of drug accountability at relevant visits as outlined in section 6.6.

6.4.5 Concomitant Care

Standard Care

Patients will be managed according to the BTS/SIGN158 British guideline on the management of asthma (https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma). This includes the use of bronchodilators, inhaled corticosteroids and theophylline. Participants may receive leukotriene receptor antagonists but not biologics. Acute asthma attacks may be managed with oral corticosteroids and antibiotics as appropriate. Patients will be withdrawn from the study if they undergo nasal polypectomy.

Concomitant medication

Patients must be receiving stable treatment for asthma and nasal disease related to N-ERD for at least 4 weeks prior to randomisation and not have any planned amendments for at least 4 weeks post-randomisation.

All concomitant medication including over the counter and herbal remedies will be recorded at baseline with any changes during participation recorded.

Non-permitted medication

The following medications are not permitted due to interactions, described in the SPC, with n-3 PUFA as outlined below and thus patients are **excluded** from the study (see section 6.3.1.3):

- None

Omega-3 ethyl ester 90 should be used with caution in patients with known sensitivity or allergy to fish.

Increased monitoring

Concomitant medication requiring increased monitoring:

- Warfarin – monitored for increase in international normalised ratio (INR) and prothrombin time.

Omega-3 ethyl ester 90 as Omacor has been given in conjunction with warfarin without haemorrhagic complications. However, the prothrombin time must be checked when Omega-3 ethyl ester 90 is combined with warfarin or when treatment with Omega-3 ethyl ester 90 is stopped.

Sites are responsible for providing increased monitoring for participants receiving the abovementioned medication(s) and for any others the PI or sub-investigator deem appropriate. Results of additional monitoring procedures do not need to be recorded on the eCRF.

6.4.6 Overdose of Trial Medication

There are no special recommendations for overdose of N-3 PUFA. Treatment should be symptomatic.

6.4.7 Protocol Treatment Discontinuation

In consenting to the trial, participants are consenting to trial treatment, 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.
- If a participant becomes pregnant, they will be advised to stop trial treatment immediately (and if 12 week dispensing was scheduled this will not take place). The participant can and should be encouraged to remain in the trial for follow up measures as far as possible.
- Withdrawal of consent for treatment by the participant.
- 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 local PI and/or CI.

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 Outcome

The primary outcome will be asthma control as assessed by change in asthma control questionnaire (ACQ)-6 mean score (http://www.qoltech.co.uk/index.htm) at 24 weeks post-randomisation of n-3 PUFA versus placebo. The ACQ-6 has six questions: five questions about symptoms and one about reliever inhaler usage. The five symptom questions are 1) woken at night by symptoms, 2) wake in the mornings with symptoms 3) limitation of daily activities 4) shortness of breath and 5) wheeze. Patients are asked to recall how their asthma has been during the previous week and to respond using a 7-point scale (0 = no impairment, 6 = maximum impairment).

6.5.2 Secondary Outcomes

The following secondary outcomes will be assessed comparing n-3 PUFA to placebo:

Asthma control

- Measured using an ACQ-7 which is the same as the ACQ-6 (above) with the addition of FEV1 % predicted (http://www.qoltech.co.uk/index.htm) at baseline 12 and 24 weeks.

General Health Related and Disease Specific Quality of Life:

Participants to complete the following questionnaires at baseline and 12 weeks and 24 weeks post-randomisation to assess outcomes listed:

- Asthma related quality of life assessed by the Mini Asthma Quality of Life Questionnaire (mini-AQLQ) will be measured at baseline, 12 weeks and 24 weeks(26). The minimum clinically important difference in scores is 0.5.
- Health-related quality of life indicated by the EQ5D-5L questionnaire to estimate quality adjusted life years(27).
- Sino-nasal disease related quality of life as assessed by the Sino-Nasal Outcomes Test (SNOT)-22(28).

Lung and Nasal Function:

- Peak nasal inspiratory flow (PNIF) will be captured every six weeks throughout the study at home (29) (where possible).
- Peak expiratory flow will be captured every six weeks throughout the study at home.
- FEV_1 and forced expiratory flow at 50% of vital capacity (FEF_{50}) will be obtained from spirometry, measured post-bronchodilator according to current American Thoracic Society/European Respiratory Society Taskforce guidelines(30). It will be measured at baseline, 12 weeks and 24 weeks (where possible). It will be reported as part of the ACQ-7 and as a separate secondary outcome.

Type 2 airway inflammation

- Fraction of exhaled nitric oxide (FeNO) will be measured, at baseline, three months and six months, using a NIOX MINO nitric oxide analyser (Aerocrine, Chicago, USA), according to the National Institute for Health and Care Excellence guidelines (www.nice.org.uk/guideance/dg12).
- Induced or spontaneous sputum (subgroup) will be collected at baseline and 24 weeks for percentage eosinophil count. Induced sputum will be collected as described by Pizzichini et al (31), sputum will be processed as described by Pizzichini et al(32) for analysis of SPM. Total cell count will be calculated and cell viability determined. Cytospins will be prepared and stained by Wright's stain for differential cell count.
- Full blood count will be measured at baseline, 12 weeks and 24 weeks for assessment of the concentration of eosinophils.

Cyclooxygenase pathway

- A urine sample will be collected at baseline and after 24 weeks of therapy. It will be analysed for LTE4 and PGD2 by radioimmunoassay and corrected for urinary creatinine.

Adherence

- Venous blood will be collected at baseline, 12 weeks and 24 weeks for assessment of red blood cell fatty acid concentration. In the situation that venous blood is not available, red blood cell fatty acid concentration will be obtained from a finger prick blood sample, which will be collected using a dried blood spot sample card and mailed to the Norwich Research Park Biorepository.

- Food frequency questionnaire (FFQ) will be completed at baseline and 24 weeks using the validated European Prospective Investigation of Cancer (EPIC) FFQ(33). The FFQ will be analysed using the online McCance and Widdowson's composition of foods integrated dataset, in order to establish habitual dietary intake, and in particular EPA and DHA.

Specialised pro-resolving mediators (SPM)

- Induced or spontaneous sputum (subgroup) will be collected at baseline and 24 weeks for SPM. Sputum samples will be acidified, applied to solid-phase extraction cartridges and the eluted SPM will be analysed by liquid chromatography–tandem mass spectrometry as described by Barden et al (34).

Asthma attacks and safety:

- Asthma attacks will be defined as those resulting in death, hospitalisation, A&E attendance, out-of-hours medical contact, or a course or boost in oral corticosteroids (prednisolone) of at least 3 days for asthma.
- Adverse events will be recorded at each study visit following randomisation.
- Full blood count, renal and liver function tests, and coagulation for those receiving warfarin will be measured in local laboratories at baseline, 12 weeks and 24 weeks.

6.6 Participant Timeline

Figure 2. Schedule of Assessments

	Screening[1]	Baseline[1]	Randomisation	6 weeks (+/- 1 week)	12 Weeks (+/- 2 weeks)	18 Weeks (+/- 2 weeks)	24 Weeks (+/- 4 weeks)
Informed Consent	X						
Eligibility	X						
Demographics, medical history and patient characteristics collected		X					
Urine pregnancy testing for WOCBP		X			X		
Randomisation			X				
IMP dispensed			X		X		
IMP adherence					X		X
ACQ-6	X	X		X	X	X	X
PNIF (where possible)		X		X	X	X	X
Peak expiratory flow		X		X	X	X	X
Spirometry		X			X		X

AQLQ		X			X		X
EQ-5D-5L		X			X		X
FFQ		X					X
SNOT-22		X			X		X
FeNO		X			X		X
Venous blood for[2] **FBC (Eosinophils)** **RBC fatty acid (dried blood spot sample)** **Safety – U&E, LFTs Clotting**		X			X		X
Urine sample –for **LTE4** **PGD2**	X (uLTE4 only)	X					X
Sputum – induced or spontaneous[3] for **Differential cell count** **SPM**		X					X
Asthma attacks			X				

Adverse events				X	X	X	X

[1] Patients can be re-screened as required.
[2] 15ml blood to be taken for each safety and biomarker analyses at all study visits.
[3] only for those consenting to the sputum sub-study.

NB where study assessments are completed within 28 days of randomisation for baseline or within the timeframes specified above, these observations can be recorded at the relevant time point to avoid patients having to repeat assessments unnecessarily provided they adhere to the requirements of this protocol.

6.6.1 Participant Assessments

6.6.1.1 Visit settings

The study requires venous blood sampling, spirometry, FeNO assessment and, in a subgroup, sputum acquisition and therefore needs to be undertaken in clinical research or other appropriate facilities. Questionnaires will be completed online, or on paper, at home or in another suitable setting and PEF undertaken at home and the results captured electronically, or on paper, at home.

6.6.1.2 Participant Correspondence

Participant correspondence will be via the local research team.

6.6.1.3 Screening

The PI or delegate is responsible for identifying eligible patients to invite to participate in the study in accordance with the eligibility criteria defined in section 6.3.1. Patient's aspirin/NSAID tolerance/N-ERD status will be reviewed and classified as (1) clear history of N-ERD, (2) high probability of diagnosis of N-ERD or (3) clear aspirin/NSAID tolerant. Eligible patients (1) or probably eligible patients (2) will be approached in clinic or identified via local patient lists or clinical records and provided with the relevant study specific literature explaining the aims, methods and potential hazards and benefits of participation in the trial (see section 6.8.1) either in person or by mail.

Patients will be given adequate time to consider their participation prior to the site seeking written informed consent electronically or paper-based following consultation in person or via phone/video call. Informed consent will be sought prior to completion of any assessments/procedures etc. required by the study outside the remit of usual care for patients in the same situation.

The potential participants will be informed prior to consenting that recruitment to the trial is dependent on the results of their screening assessments, including blood tests and a urine and/or nasal aspirin challenge test if required.

6.6.1.4 Baseline

Once written informed consent has been obtained, either in person, electronically or remotely via video/phone, the patient will be required to complete the following baseline assessments at home electronically or sent by post and returned to the site research team: ACQ-6, PEF, AQLQ, SNOT-22 and food frequency questionnaires. Patient demographics, smoking status (including vaping), alcohol intake, co-morbidities and relevant medical history (including asthma attack frequency over the last 12 months) and concomitant medication (including over-the-counter medication) will be captured by questionnaires and from the medical notes where required and recorded in the eCRF.

Most questionnaires can be completed on paper or electronically, however the ACQ-6 and AQLQ can only be completed on paper. During the consent process participants will be offered the option to complete the other questionnaires electronically. Where possible, participants will complete relevant questionnaires electronically via REDCap at each study time point. Electronic questionnaires may be emailed to the participant's personal email address for completion by those opting to provide responses electronically. They may be completed at home or using computing facilities at the site. Participants opting not to receive the questionnaires electronically and for the ACQ-6 and AQLQ, paper copies will be provided. They may complete the questionnaires at the site and hand them to the research staff or complete them at home and return to the NCTU by freepost, courier or pre-paid envelopes.

Participants who meet the initial screening criteria will be invited to attend a study visit for assessment of weight and height, FeNO, and spirometry. Blood samples will be taken for safety (full blood count (FBC), urea and electrolytes (U&Es), liver function tests (LFTs), coagulation screen) and research (RBC fatty acid concentration) purposes at the recruiting site prior to enrolment. Participants who are WOCBP will also be required to have a urine pregnancy test conducted specifically for the trial. All participants will be asked to provide a sample of urine which will be analysed for LTE4 and PGD2 (details in section 6.6.2.2). Where the abovementioned assessments and/or data is captured as part of standard care within 28 days of randomisation within the requirements of this protocol, these observations may be recorded as the baseline values to avoid patients having to repeat procedures unnecessarily.

For those meeting the inclusion criteria other than a confirmed diagnosis of N-ERD but a high probability of being aspirin/NSAID intolerant (group 2 shown in figure 3 below) an aspirin challenge will be undertaken at a screening visit and those positive will also be eligible for study recruitment (see section 6.8.1).

Participants consenting to the sputum sub-study at applicable sites will also be required to undergo induced sputum sampling according to previously described protocols(31). A spontaneous sputum sample may be considered an acceptable method of collecting sputum for all patients at a site if COVID-19 restrictions render induced sputum sampling unacceptable. Induced sputum sampling will be undertaken on a separate visit to the aspirin challenge (if required).

6.6.1.5 Randomisation

Upon completion of and when results of all baseline assessments are known, site staff delegated the appropriate responsibility will have the option to randomise the patient. Treatment allocation will be determined by a computer-generated randomisation code via a web-based system facilitated by NCTU. A semi-blinded randomisation outcome will be circulated via email to the site clinical trial pharmacy only. The site PI and delegates will receive a blinded randomisation notification email.

An initial 3-month supply of IMP will be dispensed by the site pharmacy following confirmation of treatment allocation and receipt of a completed trial prescription signed by the PI or delegated sub-investigator. The site pharmacy and research team are responsible for ensuring the participant receives their capsules at an appropriate clinic visit or in exceptional circumstances via courier after liaising with NCTU and receiving approval of the CI.

The participant's General Practitioner (GP) will be informed of their randomisation into the study.

6.6.1.6 Six Week Assessment

The following assessments should be completed at home at 6 weeks post-randomisation: ACQ-6, PEF, adverse event questionnaire and concomitant medication questionnaire. Site staff will contact participants by phone/video call to assess adverse events if they have been flagged on the questionnaire.

Where these assessments are completed as part of standard care and within the time frame permitted by and requirements of this protocol, the results may be recorded for those required at the 6-week study time point.

Participants completing paper-based questionnaires will be provided with a freepost envelope to return them to the trials unit otherwise they will be completed electronically via REDCap.

This assessment may take place +/- 1 week of the scheduled date.

6.6.1.7 12 Week Study Visit

The following assessments should be completed 12 weeks post-randomisation: ACQ-6, PEF, PNIF, AQLQ, SNOT-22, spirometry, FeNO, RBC fatty acids and blood for eosinophils and safety monitoring and urine pregnancy test for WOCBP. Site staff will assess adverse events (including asthma attack frequency) and any changes to concomitant medication and/or medical history.

IMP adherence will be measured by a pill count completed by site staff/participant during the visit. This may be conducted at site where visits take place in person or participants will be asked to confirm the number of remaining capsules via phone/video call. If the participant has reduced their trial treatment dose due to adverse reaction(s) or intolerability this should be documented in the eCRF and a new dosing card administered. Participant should return the superseded dosing card either in person or via freepost envelope.

Participants completing paper-based questionnaires will be provided with a freepost envelope to return them to the trials unit otherwise they will be completed electronically via REDCap.

Where any of these assessments are completed as part of standard care and within the time frame permitted by and requirements of this protocol, the results may be recorded for those required at the 12 week study time point.

Participants will be supplied with a final 12 week supply of IMP (5 bottles whether on full or reduced dose) as prescribed by the PI or delegate, dispensed by the site clinical trial pharmacy. Site staff to ensure patient is administered dosing card with correct treatment schedule. The site pharmacy and research team are responsible for ensuring the participant receives their capsules at this clinic visit.

This visit may take place +/-2 weeks of the scheduled date.

6.6.1.8 18 Week Assessment

The following assessments will be completed 18-weeks post-randomisation: ACQ-6, PEF, adverse event questionnaire and concomitant medication questionnaire. Site staff will contact participants by phone/video call to assess adverse events (including asthma attack frequency) if they have been flagged on the questionnaire.

Where these assessments are completed as part of standard care and within the time frame permitted by and requirements of this protocol, the results may be recorded for those required at the 18-week study time point.

Participants completing paper-based questionnaires will be provided with a freepost envelope to return them to the trials unit otherwise they will be completed electronically via REDCap.

This assessment may take place +/- 2 weeks of the scheduled date.

6.6.1.9 24 Week Visit

The following assessments should be completed 24 weeks post-randomisation: ACQ-6, PEF, PNIF, AQLQ, SNOT-22 and food frequency questionnaires, spirometry, FeNO, urine sample, RBC fatty acids, induced or spontaneous sputum (subgroup) and blood for eosinophils and safety monitoring. Site staff will assess adverse events (including asthma attack frequency) and any changes to concomitant medication and/or medical history.

IMP adherence will be measured by a pill count completed by site staff/participant during the visit. This may be conducted at site where visits take place in person or participants will be asked to confirm the number of remaining capsules via phone/video call. If the participant has reduced their trial treatment dose due to adverse reaction(s) or intolerance this should be documented in the eCRF. Participant should return their dosing card either in person or via freepost envelope.

Participants completing paper-based questionnaires will be provided with a freepost envelope to return them to the trials unit otherwise they will be completed electronically via REDCap.

Participants consenting to the sputum sub-study at applicable sites will also be required to undergo induced sputum sampling according to previously described protocols(31). A spontaneous sputum sample may be considered acceptable method of collecting sputum for all patients at a site if COVID-19 restrictions render induced sputum sampling unacceptable.

Where any of these assessments are completed as part of standard care and within the time frame permitted by and requirements of this protocol, the results may be recorded for those required at the 24-week study time point.

This visit may take place +/- 4 weeks of the scheduled date.

6.6.2 Human Tissue Samples

6.6.2.1 Blood

15ml of whole blood will be taken for safety at baseline, 12 and 24 weeks post-randomisation. These may be taken and processed at the recruiting site.

15ml of whole blood for research purposes will be taken at baseline, 12 and 24 weeks post-randomisation at the recruiting site where possible. Where study visits are conducted remotely, patients will receive a home-kit for collection of a blood sample from a finger prick using a dried blood spot sample card and they will return it by post to Bioanalytical facility, Bob Champion Research and Education Centre. However, it may not be possible to obtain samples from all participants at any or both timepoints. Zero, one or both research blood samples will be obtained from participants willing to provide samples for analysis in future research projects.

If participants are willing, we will also ask to take an additional blood sample of 15ml for research purposes, which will be analysed in future ethically approved studies. This may include genetic analysis if the participants consent to this. These samples will be stored at the Norwich Research Park Biorepository at the end of the study.

6.6.2.2 Urine

Participants will be asked to provide a urine sample for research purposes at screening, baseline and at 24 weeks post randomisation. For consistency the baseline and screening samples should be collected at the same time of day.

Screening sample

The participant will be asked to collect a sample ideally between 8-10am which will be transported to the Bioanalytical facility, Bob Champion Research and Education Centre, Norwich Medical School direct from the participants home or via the hospital.

Baseline and 24 weeks post-randomisation sample

Ideally the sample should be of the second elution of the day collected between 0800 and 1000 on the day of the study visit as previously described(35) although, as there is no evidence of diurnal variation(36), the timing of the sample is not crucial. However, sample collection should be the same time of day for both the baseline and 24 week samples. Ideally sample collection should be undertaken in the morning at the hospital clinic/research facilities but if that is not possible it can be collected at an afternoon study visit. The sample will then be aliquoted into 3 x 1ml pre-labelled vials and stored at -80 C before batch shipping on dry ice to the Bioanalytical facility, Bob Champion Research and Education Centre.

6.6.2.3 Sputum

Sputum will be collected, processed and analysed according to the sputum laboratory manual. In brief, induced sputum will be collected as previously described(31) after inhalation of increasing concentrations of hypertonic saline if possible. Sputum will be processed as described by Pizzichini et al(32). The sputum plugs will be selected from saliva, processed with dithiothreitol (DTT) and centrifuged. Supernatants will be stored at -80^{o}C for analysis of SPM. Total cell count will be calculated and cell viability determined. Cytospins will be prepared and stained by Wright's stain for differential cell count. For SPM, sputum samples will be acidified, applied to solid-phase extraction cartridges and the eluted SPM will be analysed by liquid chromatography–tandem mass spectrometry as previously described(34). This analysis will be undertaken by trained technicians in centres with experience of undertaking, or capacity to undertake, this analysis for clinical research.

6.6.2.4 Storage and transportation

The lab manual will provide further details of sample handling and storage at recruiting sites, and transported to the Bioanalytical facility, Bob Champion Research and Education Centre for analysis then storage at Norwich Research Park Biorepository for future analysis of any potential biomarker including but not limited to N-ERD, asthma, or nasal disease pending additional ethical approval, at a later date.

Sample handling training will be provided to all sites prior to activation detailing sample collection, handling and storage procedures. Detailed written instructions and appropriate tissue transfer agreements will be put in place prior to the transfer of relevant material.

6.6.3 Early Stopping of Follow-up

Sites must inform NCTU of all forms of early trial discontinuation via the eCRF. In instances where a participant has decided to withdraw consent, it is essential for the site to establish which aspects of the trial the participant is withdrawing consent from.

- 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 provide data either through study visits or remotely via the registry, this view must be respected, and the participant withdrawn entirely from the trial. 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. Any samples provided by the participants, which have yet to be analysed will be destroyed and the registry will be updated so that no further data is provided to NCTU.

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 local PI and/or CI.

Participants who stop trial follow-up early will not be replaced.

6.6.4 Participant Transfers

If a participant moves from the area making continued follow-up at their consenting centre inappropriate, every effort should be made for them to be followed-up at another participating trial centre. Written consent should be taken at the new centre, either electronically or paper-based following consultation either in person or via video/phone call, and then a copy of the participant's CRFs should be provided to the new centre. Responsibility for the participant remains with the original consenting centre until the new consent process is complete.

6.6.5 Loss to Follow-up

Sites will be asked to account for the vital status and details of admission to hospital for all patients who have consented to participate in the study regardless of whether they have withdrawn from the intervention or study assessments.

6.6.6 Trial Closure

The end of the trial is defined as 4 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

The primary outcome will be the change in asthma control questionnaire (ACQ-6) mean score. This has a standard deviation (SD) of 0.67(37) and a minimal important difference of 0.5(38). Assuming a 20% withdrawal rate, we will have 90% power at the 0.05 significance level to detect a change in our primary outcome if we randomise 98 individuals into the study. Assuming a 30% withdrawal rate, a sample of 52 patients will provide 80% power to detect a 15% reduction in absolute sputum eosinophil count(39). The DMC will monitor the assumptions of the sample size calculation in accordance with the Terms of Reference.

6.8 Recruitment and Retention

6.8.1 Recruitment

Patients will be identified from review of patient registries, hospital medical records and databases of research interested patients or clinical details as well as those who self-refer. However, many people with N-ERD are undiagnosed as the proportion of people with N-ERD recorded in clinical records do not equate to detailed studies. Targeted screening of people with a high probability of a diagnosis of N-ERD is permitted within the protocol. The screening and recruitment strategies, summarised in figure 3, may include any of the following:

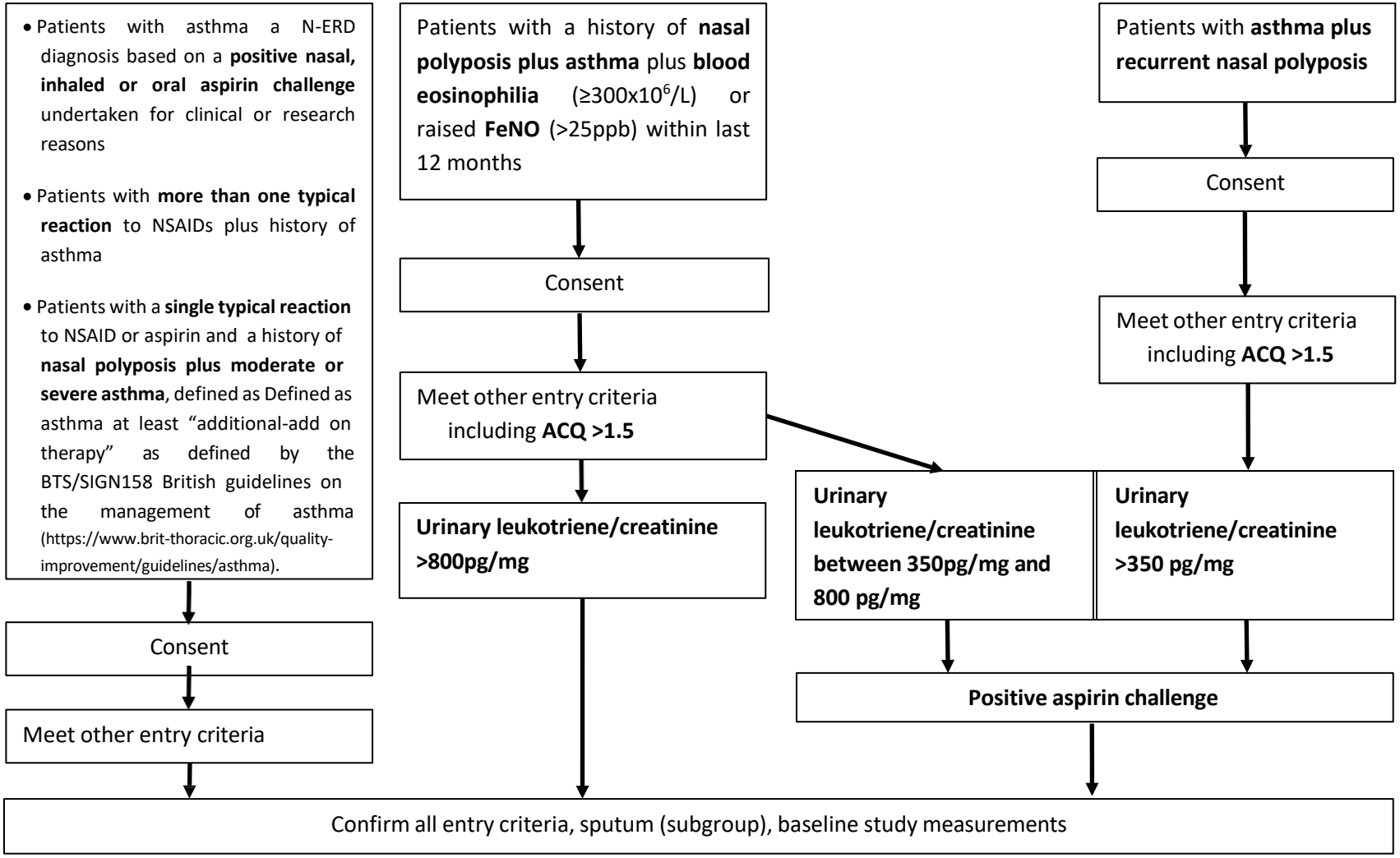
- Patients with asthma and a clear diagnosis of N-ERD based on a previous positive nasal, inhaled or oral aspirin challenge undertaken for clinical or research reasons will be considered to have a **clear diagnosis** of N-ERD and will meet this eligibility criteria for participation in the study.
- Patients with more than one reaction to NSAIDs plus history of nasal polyposis or asthma will be considered to have a **clear diagnosis** of N-ERD and will meet this eligibility criteria for participation in the study.
- Patients with a single reaction to a NSAID or aspirin plus a history of **nasal polyposis plus moderate or severe** asthma, defined as, asthma at least "additional-add on therapy" as defined by the BTS/SIGN158 British guidelines on the management of asthma (https://www.brit-thoracic.org.uk/quality-improvement/guidelines/asthma), and one the following criteria will be considered to have a **clear diagnosis** of N-ERD and will be invited to participate in the study.
- Patients with a history of nasal polyposis plus asthma plus blood eosinophilia (≥300x106/L) or raised FeNO (>25ppb) within last 12 months will be considered to have a high probability N-ERD and will be enrolled in the study if they have a uLTE4/Creatinine concentration of greater than 800pg/mg(35, 40).
- Patients with asthma and a history of nasal polyposis will be considered to have a high probability of N-ERD and can be enrolled into the study if they have a uLTE4/Creatinine concentration of greater than 350 pg/mg up and a positive nasal aspirin challenge as described below.

Nasal aspirin challenge will be modified from the published version of the aspirin challenge(41) to reduce the dose steps as this is appropriate for a screening test for the purpose of this study. One sachet (500mg) of lysine aspirin will be dissolved in 10 ml saline. Peak nasal inspiratory flow or acoustic rhinometry plus evaluation of upper and lower respiratory tract symptoms will be undertaken before and 10 minutes after nasal instillation of saline and 45 minutes after nasal instillation of 15mg (0.15ml in each nostril) lysine aspirin followed by 30mg (0.3ml each nostril) then 100mg (1ml in each nostril) of lysine aspirin. The test will be considered positive at any stage if there are symptoms plus a greater than 25% reduction in nasal volume or a 50% reduction in peak nasal inspiratory flow. If this occurs post saline the subject is hyper-reactive and the test should be stopped and re- performed once better control of upper airway inflammation is achieved.

Patients will be approached by the clinical care team, by way of any of the following methods, if they have documented history of a clear diagnosis of N-ERD or high probability of N-ERD and will be invited to participate in the study and attend a screening visit to establish their suitability in terms of the other entry criteria. The clinic staff will arrange a subsequent screening visit.

- The clinic team may mail (by post or e-mail) an invitation letter with or without a participant information sheet along with a contact email address/phone number to respond to, detailing a range of methods for the interested potential patients to contact the local trial team to arrange a screening appointment.
- Where patients are due to attend clinic for a routine appointment in the near future, the clinical care team may mail (by post or e-mail) an invitation letter on hospital headed paper which provides an overview of the study, and a participant information sheet, so that the patient receives these documents at least 24 hours in advance of the forthcoming routine clinic assessment visit. After the participant has provided written informed consent, screening for eligibility and baseline assessments will be undertaken at the routine clinic visit.
- For centres with access to a volunteer database, the researchers may mail the invitation letter and reply form directly to the volunteer.

Figure 3: PUFFIN Identification and Recruitment Strategies



Potential patients may be contacted by phone between 3 and 7 days after the mailing of the letter/sending the email to ensure that they have received it. Enrolment will then be scheduled either via a face-to-face visit or by consultation via phone/video call for those willing to consent to participate. Potential participants and site staff will confirm whether consent will be recorded electronically or on paper, with the decision documented in the patient's medical notes, to facilitate preparations for the enrolment visit.

Remote consent

For those being enrolled remotely and completing paper consent forms, a localised consent form with a freepost or stamped address envelope, will be mailed to the potential participant for their completion following the study consultation. The person taking consent will countersign the form upon receipt at the recruiting site.

Where electronic consent is being sought, participants will verbally consent to the sharing of their email address and/or contact details in order to receive the link to the electronic consent form to facilitate the process. This will be documented by site staff in the patient's medical notes. Both parties will complete the electronic consent form following the phone/video call consultation by providing a wet-ink signature equivalent on the designated field via REDCap (49).

Details of the consultation, including but not limited to the time/date of the phone/video call, confirmation of identification checks and the name of the staff member leading the call and thus taking consent will be recorded in the patient's medical notes. Copies of the completed forms will be mailed (by post or email) to participants.

6.8.2 Retention

This study has been designed to be of a short duration and to utilise remote assessments wherever possible. It is intended that questionnaires are completed at home to minimise the time that the participants are in the hospital. In exceptional circumstances drug may be able to be couriered to the patients for convenience; however, this will need to be arranged via the NCTU Trial Team with approval from the CI. However, some of the study assessments require physiological measurements required to be undertaken in a clinical research facility or similar setting.

Participants will also be offered the option to subscribe to an email-based participant newsletter during the study's recruitment period in order to help keep participants engaged and informed of study progress during and beyond their participation.

Participants will be given/posted a card with the contact details of the local PI that will request details of hospital admissions to be reported at consent/baseline. Patients will be asked to provide informed consent for their contact details to be stored in a trial contacts database at UEA. Participants can withdraw consent to this in writing at any time.

6.9 Assignment of Intervention

6.9.1 Allocation

6.9.1.1 Sequence generation

The allocated treatment for a patient will be generated via computer written code using stratification. Stratification will be performed using the factors measured at baseline: i) study site, ii) participation in the sputum sub-study (yes/no)).

Full details of the stratification will be documented in a separate document (PUFFIN Allocation Schedule) stored in a shared file accessible to only the study statistician(s) and data management team as appropriate.

6.9.1.2 Allocation concealment mechanism

Allocation will be computer generated by a web-based system ensuring concealment prior to randomisation. Following consent and confirmation of eligibility the PI or delegate will enter data confirming eligibility into the eCRF generating a participant identification number. Blinded notification of randomisation will be sent to the PI and/or delegates, CI and NCTU trial team. A semi-blinded notification of randomisation will be sent to the site clinical trial pharmacy.

6.9.1.3 Allocation Implementation

The PI or delegated sub-investigator is responsible for ensuring only eligible patients are randomised and prescribed study medication. Patients will be allocated to the intervention by a process embedded in the web-based data management system. The randomisation code will be saved in the study database for later decoding and also for emergency unblinding purposes.

6.9.2 Blinding

This is a double-blind study. The placebo and active treatments will appear identical and will be dispensed in identical containers. All trial participants, care providers and outcome assessors will remain blind throughout the study.

6.9.3 Emergency Unblinding

The decision to unblind a single case should be made when knowledge of an individual's allocated treatment is required:

- To enable treatment of severe adverse event/s, or
- In the event of an overdose.

Where possible, requests for emergency or unplanned unblinding of individuals should be made via the trial manager and agreement of the CI will then be sought. However, in circumstances where there is insufficient time to make this request or for agreement to be sought, the treating clinician should make the decision to unblind immediately. This will be done via the study database (local PIs and the CI will have special logins which will allow unblinding and which will be closely audited within the database management system) or by contacting the CI who will authorise unblinding by the Data Management Team. All instances of unblinding should be recorded and reported to NCTU by the local PI, or delegate, including the identity of all recipients of the unblinding information.

6.9.4 Non-emergency Unblinding

For circumstances where non-emergency unblinding is required e.g. GP/clinician or participant request, following confirmation from the CI, treatment allocation will be revealed by the eCRF, using

logins and forms separate to those required to unblind in emergency situations. The blinding will be maintained for as many of the local study and NCTU trial team members as possible. A record of non-emergency unblinding and those individuals unblinded to trial treatment will be recorded and filed.

6.10 Data Collection, Management and Analysis

6.10.1 Data Collection Methods

An on-line patient screening log will contain the details of the patient initials, date of contact and the route of identification for all potential participants who have received a patient information sheet (PIS). Following confirmation of consent, each participant will be given a unique trial Participant IDentification Number (PID). Data will be collected at the time-points indicated in the Schedule of Assessments (see section 6.6).

Data collection will vary according to the data variable in question.

1) ACQ is recognised by the American Thoracic Society and NICE and widely used in research (http://www.qoltech.co.uk/index.htm). The ACQ-7 has seven items: five questions about symptoms, one about reliever inhaler usage and a measure of airflow. The five symptom questions enquiring are 1) woken at night by symptoms, 2) wake in the mornings with symptoms, 3) limitation of daily activities, 4) shortness of breath and 5) wheeze. Patients are asked to recall how their asthma has been during the previous week and to respond using a 7-point scale (0 = no impairment, 6 = maximum impairment). Clinic staff score the FEV1% predicted on a 7-point scale. ACQ-7 score is the mean of the 7 questions and therefore between 0 (totally controlled) and 6 (severely uncontrolled). The ACQ-7 is validated using FEV1 and although sometimes PEF is used in its place, this is not recommended. The ACQ-6 (ACQ-7 without lung function assessment) is also validated and reflects the weekly impact of asthma on the individual. We have chosen the ACQ-6 (symptoms and beta agonist usage) as this does not require measurement of spirometry, which is considered an aerosol generating procedure, and there is high agreement between the different versions of the ACQ (37). It will be measured every 6 weeks.

2) Asthma Quality of Life Questionnaire.

Mini Asthma Quality of Life Questionnaire (mini-AQLQ) will be measured at baseline, 12 weeks and 24 weeks. It is a 15-item questionnaire as is a short version of the complete 32-item questionnaire. It comprises four domains (symptoms, environment, emotions, activities) and assess the previous 2-week period. Scores range from 0-6 (lower is worse). The mini-AQLQ score is calculated as the average of domain items(26). The minimum clinically important difference is 0.5.

3) Health related quality of life.

The EQ-5D-5L(27) will be measured at baseline, 12 weeks and 24 weeks. It will be converted to utilities using standard UK health state valuations(42) and thence to Quality Adjusted Life Years (QALYs) gained by evaluation at multiple time-points and calculating the area under the curve over a 6-month period.

4) Airway calibre.

FEV_1 and FEF_{50} will be obtained from spirometry, measured post-bronchodilator according to current American Thoracic Society/European Respiratory Society Taskforce guidelines(30). It will be measured at baseline, 12 weeks and 24 weeks (where possible). It will be reported as part of the ACQ-7 and as a

separate secondary outcome. The percentage predicted value will be taken from the reference values of the Global Lung Function Initiative (GLI) equations(43). All personnel undertaking this procedure will be accredited by the Association for Respiratory Technology & Physiology (ARTP) or will receive specific training at site initiation. Spirometry is considered to be the gold standard assessment of airway calibre and bronchoconstriction in airways disease and is fundamental in the assessment of patients with asthma. It is undertaken as part of routine clinical practice. Peak expiratory flow (PEF) will be measured every 6 weeks at home by the participants.

5) Peak nasal inspiratory flow (PNIF) is a method of assessing upper airway calibre. It will be measured by an In-Check Nasal Inspiratory Flow Meter (Clemment Clarke International Ltd, Harlow, UK). It correlates highly to nasal symptoms scores(44). It will be measured every 6 weeks at home (where possible).

6) FeNO will be measured, at baseline, 12 weeks and 24 weeks, using a NIOX MINO nitric oxide analyser (Aerocrine, Chicago, USA), according to the National Institute for Health and Care Excellence guidelines (www.nice.org.uk/guideance/dg12). It is used routinely in clinical care and recommended by NICE (NG80). FeNO is higher in people with N-ERD than aspirin tolerant asthma and increases to a significantly higher degree following aspirin challenge(45).

7) Urinary LTE4 and PGD2.

A urine sample will be collected at baseline and after 24 weeks of therapy and analysed by radioimmunoassay. uLTE4 reflects 4-series leukotriene pathway and is a biomarker for N-ERD. It will be corrected for urinary creatinine as this standard practice for this metabolite. PGD2 will also be measured using radioimmunoassay and will used to assess the cyclooxygenase pathway. PGD2 was also reduced significantly in the pilot study(46). See section 6.6.2.2 for details about sample collection, storage and transport.

8) Red blood cell fatty acid concentration (to assess adherence and fatty acid status - biological response relationships).

This will be measured at baseline, 12 weeks and 24 weeks. RBCs will be used as the blood lipid pool to assess habitual PUFA status at baseline and in response to intervention. In contrast to plasma fractions such as plasma cholesterol ester or triglycerides, which are variable and reflect recent intakes (last meal up to two weeks), RBC are a tissue which provide a marker of habitual intake in the few months(47) with less biological variability than plasma PUFA pools. For the RBC an increase of approximately 3.2-fold would be predicted following a 4g Omega-3 ethyl ester 90 dose(48). For the sensitivity analysis a 75% adherence cut-off will be used which equates to an RBC EPA+DHA enrichment of 2.7-fold.

9) The differential cell count and concentration of SPMs in sputum (subgroup of 52 patients at baseline and 24 weeks).

Induced sputum will be collected as previously described(31) after inhalation of increasing concentrations of hypertonic saline if possible. Sputum will be processed as described by Pizzichini et al(32). The sputum plugs will be selected from saliva, processed with dithiothreitol (DTT) and centrifuged. Supernatants will be stored at -80^{o}C for analysis of SPM. Total cell count will be calculated and cell viability determined. Cytospins will be prepared and stained by Wright's stain for differential

cell count. For SPM, sputum samples will be acidified, applied to solid-phase extraction cartridges and the eluted SPM will be analysed by liquid chromatography–tandem mass spectrometry as previously described(34).

10) Blood eosinophils.

These will be measured, at baseline, 12 weeks and 24 weeks, in local clinical laboratories using coulter counters.

11) Full blood count, renal and liver function tests, and coagulation. These will be measured in local laboratories at baseline, 12 weeks and 24 weeks.

12) Urine pregnancy tests (WOCBP only) will be measured at baseline and 12 weeks.

13) Food frequency questionnaire (FFQ).

This will be performed at baseline, and 24 weeks using the validated European Prospective Investigation of Cancer (EPIC) FFQ(33), with the FFQ recognised as a robust methodology to capture habitual diet and is particularly suited to quantify foods not consumed on a daily basis, such as fish (main dietary source of EPA and DHA). The FFQ will be analysed using the online McCance and Widdowson's composition of foods integrated dataset, in order to establish habitual dietary intake; in particular, EPA and DHA.

14) Asthma attacks.

Asthma attacks will be defined as those resulting in death, hospitalisation, A&E attendance, out-of-hours medical contact, or a course or boost in oral corticosteroids (prednisolone) for asthma. The number of asthma attacks over the duration of the study will be captured.

6.10.2 Data Management

Participants will complete questionnaires online or on paper, depending on the licencing rules of the questionnaires AACQ-6 and ACLQ must be completed on paper) and the participant's preference. Paper questionnaires will be sent to NCTU (by post or email if returned to sites) where they will be transcribed into the eCRF. Online responses will be stored directly in the eCRF.

Positive tests for COVID-19 during trial participation will be recorded on the eCRF following a review of patient medical notes/discussion with participants by site staff. COVID-19 infection testing will only be carried out as part of local standard care testing where required, there are no trial specific requirements. All local guidelines will be followed with regards to COVID-19 safety measures.

Further information on data collection and management processes are provided in the PUFFIN Data Management Plan.

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 (see Section 6.10.2).

Data collection, data entry and queries raised by a member of the PUFFIN trial team will be conducted in line with NCTU and trial specific Data Management Standard Operating Procedure.

Identification logs and enrolment logs will be kept at the trial site in a locked cabinet within a secured room.

Participant identifiable data will be stored on a Participants Database for the purpose of contacting participants to collect trial data etc., sending questionnaires and reminders and for sending newsletters during the trial. All participant identifiable data will be stored securely, with access only granted to those members of the study team who require it.

Clinical trial team members will receive trial protocol training. All data will be handled in accordance with the Data Protection Act 2018 and GDPR 2018 and any subsequent revisions.

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 PUFFIN trial team at NCTU, PUFFIN study teams at sites 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 eCRF and associated code will be developed by NCTU Data Management, in conjunction with the PUFFIN trial team. The eCRF software will provide 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 eCRF will be retained for at least 25 years on the servers of NCTU for on-going analysis of secondary outcomes.

The identification 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 forms on hospital computers. After completion of the trial the identification and enrolment logs will be stored securely by the sites for 25 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 through capsule counts of unused returned drug supplies at each relevant study visit. We will consider 80% adherence as required for inclusion in the per protocol analysis.

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 local PI and/or CI.

6.10.4 Statistical Methods

6.10.4.1 Outcomes

Primary outcome

The primary outcome, change in ACQ -6 mean score six months post-randomisation, will be analysed using a general linear model adjusting for stratification variables.

Secondary outcomes

ACQ-7, AQLQ, EQ5D-5L, FEV_1, FEF_{50}, SNOT-22 at 12 weeks and 24 weeks post randomisation will be analysed using a general linear model adjusting for stratification variables.

Lung function data will be analysed using a repeated measures model.

Eosinophil count at 12 weeks and 24 weeks post randomisation will be analysed using a general linear model adjusting for stratification variables.

EPA and DHA, estimated from the FFQ, will be analysed using a general linear model adjusting for stratification variables.

The number of asthma attacks will be analysed using a Poisson regression model adjusting for stratification variables.

The assumptions of all the models will be checked using residual analysis and, if appropriate, alternative methods will be used.

6.10.4.2 Statistical Analysis Plan (SAP)

A full SAP will be produced prior to the analysis of any data. Both the TSC and DMC 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

Adjusted analyses will be undertaken for the outcomes by adjusting for the baseline value of the respective outcome measure. The primary analysis will be that adjusting for baseline.

Additional adjusted analyses may be undertaken for factors known to be associated with the outcome as decided by the TMG prior to analysis.

A sensitivity analysis will be undertaken based on those with a RBC EPA+DHA enrichment of 2.7-fold.

Further exploratory analyses maybe undertaken, but these will be detailed in the SAP prior to analysis.

6.10.4.4 Analysis Population

The analysis population are defined as:

a) intention-to-treat (ITT): all randomised individuals analysed according to randomisation regardless of adherence.
b) If compliance is less than 85% then a pill count compliance analysis also be carried out defining compliance as taking at least 80% of study medication based on pill counts. This will be detailed in the SAP.

6.10.4.5 Missing Data

The pattern of missing data will be assessed and if appropriate, multiple imputation will be used to account for missing data under the assumption that the data are missing at random. If the data are not considered missing at random then a sensitivity analysis of the results will be undertaken by considering appropriate scenarios, such as the worst-case scenario or pattern-mixture models.

6.10.5 Analysis of Tissue Samples

A laboratory analysis plan for blood, sputum and urine will be agreed and approved by the CI and TMG prior to analysis. In addition, a laboratory manual for sample handling will be developed and agreed by the CI and circulated to sites prior to activation to recruitment.

6.11 Safety reporting

6.11.1 Safety reporting

Adverse events will be collected at each visit and analysed according to the SAP. Adverse events by treatment group will be reviewed regularly by the DMC as described in their Terms of Reference (ToR).

Definitions of harm of the EU Directive 2001/20/EC Article 2 based on the principles of ICH GCP apply to this trial.

Table 2: 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 (e.g. Investigator's Brochure for an unauthorised product or 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 (e.g. 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 e.g. elective cosmetic surgery.
- Overdose of medication without signs or symptoms.

AEs will be coded according to the current version of the Common Terminology Criteria for Adverse Events (CTCAE) dictionary.

6.11.2 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 on the adverse events form of the eCRF within 7 days of report/becoming aware.

All SARs and SAEs should be notified to NCTU immediately after the investigator becomes aware of the event (in no circumstance should this notification take longer than 24 hours).

6.11.2.1 *Seriousness assessment*

When an AE or AR occurs, the investigator or delegated sub-investigator responsible for the care of the participant must first assess whether or not the event is serious using the definition given in Table 2. If the event is classified as 'serious' an SAE form must be completed and NCTU notified immediately. Investigators will also assess causality of the event(s) according to Table 3.

6.11.2.2 *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.

6.11.2.3 *Causality*

The investigator must assess the causality of all serious events in relation to the trial treatment using the definitions in Table 3.

Table 3: Adverse Event 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 (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition 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 section 6.4.1.4 of this protocol.

6.11.2.4 *Expectedness*

If there is at least a possible involvement of the trial IMP (including any comparators), the investigator and NCTU must assess the expectedness of the event. An unexpected adverse reaction is one that is not reported in the approved SPC, or one that is more frequently reported or more severe than previously reported. See section 4.8 of the SPC for a list of expected reactions associated with the IMP being used in this trial. If a SAR is assessed as being unexpected, it becomes a suspected, unexpected, serious adverse reaction (SUSAR) and MHRA and REC reporting guidelines apply (see Notifications sections of the protocol section 6.11.3). A meta-analysis of randomised controlled trials has shown an increase in atrial fibrillation in cardiac patients who received omega-3 fatty acids compared to those who received placebo (50).

6.11.3 Procedures to follow if a participant becomes pregnant

It is unlikely that a participant will become pregnant during the trial as this is an exclusion criterion with trial specific testing and acceptable methods of contraception must be used, however WOCBP are not excluded. As soon as a member of staff is notified of a pregnancy the local PI must be informed and this will be recorded in the eCRF. The participant must be advised to stop taking the IMP immediately and no further dispensing will take place. NCTU must be notified using the specific pregnancy reporting form and where the participant is willing the outcome must be followed up for mother and child. The participant should be encouraged to remain in the study for follow up as far as possible.

Pregnancy itself does not meet the definition of an SAE and does not need to be reported as such. If the outcome of the pregnancy does meet the definition of a SAE it must be reported in the usual way.

6.11.4 Notifications

6.11.4.1 *Notifications by the Investigator to NCTU*

NCTU must be notified of all SAEs immediately and no later than 24 hours of the investigator becoming aware of the event.

Investigators should notify NCTU of any SAEs occurring from the time of consent 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 or sub-investigator (a clinician 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 NCTU SAE reporting email address:

nctu.safety@uea.ac.uk

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 redacted and replaced with trial identifiers on any test results.

6.11.4.2 *NCTU responsibilities*

Medically qualified staff at NCTU and/or the CI (or a medically qualified delegate) will review all SAE reports received and the NCTU trial team will notify the Sponsor as appropriate. 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 causality attributed to n-3 PUFA by the local investigator cannot be downgraded by other parties.

NCTU is responsible for the reporting of SUSARs to the regulatory authorities (MHRA and competent authorities of other European member states and any other countries in which the trial is taking place) and REC as appropriate. Fatal and life threatening SUSARs must be reported to the competent authorities within 7 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.

The trial manager or delegate at NCTU will submit Development Safety Update Reports (DSURs) to competent authorities.

6.12 Data Monitoring

6.12.1 Data Monitoring Committee

Details of the roles and responsibilities of the Data Monitoring Committee (DMC), including membership, relationships with other committees, decision making processes, and the timing and frequency of interim analyses (and description of stopping rules and/or guidelines where applicable) are described in detail in the PUFFIN DMC ToR.

6.12.2 Interim Analyses

There are no interim analyses planned.

6.12.3 Quality Assurance and Control

6.12.3.1 Risk Assessment

The Quality Assurance (QA) and Quality Control (QC) considerations for the PUFFIN 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.

6.12.3.2 Central Monitoring at NCTU

NCTU staff will review the eCRF 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 PUFFIN trial Data Management Plan.

Patients are consented to enable NCTU to hold a copy of the completed consent form to allow central data monitoring checks to be completed.

6.12.3.3 On-site Monitoring

The frequency, type and intensity of routine and triggered on-site monitoring will be detailed in the PUFFIN 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.

6.12.3.3.1 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.

6.12.3.4 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 PUFFIN QMMP.

6.12.3.4.1 Trial Management Team

The Trial Management Team (TMT) will be set up to assist with developing the design, co-ordination and day-to-day operational issues in the management of the trial, including budget management. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TMT terms of reference.

6.12.3.4.2 Trial Management Group

A Trial Management Group (TMG) will be set up to assist with developing the design, co-ordination and strategic management of the trial. The membership, frequency of meetings, activity (including trial conduct and data review) and authority will be covered in the TMG terms of reference.

6.12.3.4.3 Independent Trial Steering Committee

The Independent Trial Steering Committee (TSC) is the independent group responsible for oversight of the trial in order to safeguard the interests of trial participants. The TSC 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 TSC terms of reference.

6.12.3.4.4 Independent Data Monitoring Committee

The Independent Data Monitoring Committee (IDMC) is the only oversight body that has access to unblinded accumulating comparative data. The IDMC is responsible for safeguarding the interests of trial participants, monitoring the accumulating data and making recommendations to the TSC on whether the trial should continue as planned. The membership, frequency of meetings, activity (including review of trial conduct and data) and authority will be covered in the IDMC terms of reference. The IDMC will consider data in accordance with the SAP and will advise the TSC through its Chair.

6.12.3.4.5 Trial Sponsor

The role of the sponsor, Norfolk and Norwich University Hospitals NHS Foundation Trust, 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. A proportion of the Sponsor's activities have been delegated to the CI, UEA and NCTU as outlined on the form for delegated activities agreed and signed by all parties before the start of the trial.

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

This protocol will be submitted to the MHRA.

This is a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC. Therefore, a CTA is required in the UK.

The progress of the trial, safety issues and reports, including expedited reporting of SUSARs, will be reported to the MHRA in accordance with relevant requirements and practices.

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 the Sponsor, NCTU and the relevant site as required.

A copy of the local R&D approval and of the Participant Information Sheet (PIS) and consent form on local hospital 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 MHRA, HRA or Ethics Committee for categorisation and approval as required. Once the amendment has been categorised it will be sent to relevant sites for consideration in accordance with standard MHRA and/or HRA processes and timescales. Amendments must not be implemented until all approvals are received and sites have either confirmed acceptance or, no objection has been received within the defined timescale(s). Notification will be sent by NCTU to trial personnel to confirm when an amendment can be implemented.

A summary of protocol amendments will be maintained within the protocol.

7.5 Consent or Assent

Patients will be provided with a PIS and given time to read it fully. Following a discussion with a medical qualified investigator or suitably trained and authorised delegate, any questions will be satisfactorily answered and if the participant is willing to participate, written informed consent will be obtained either electronically or paper-based following consultation in person or via phone/video call. 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 participant 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 and PIS are 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 identification number 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/deleted once checks are complete. Consent forms will not be kept with any additional patient data.

Identifiable data will be shared with the NCTU trial team only following written informed consent or verbal consent from the patient to participate in the study.

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 management and conduct of the research.

7.9 Finance

This project (project reference NIHR129910) is funded by the Efficacy and Mechanism Evaluation (EME) Programme, an MRC and NIHR partnership. The views expressed in this publication are those of the author(s) and not necessarily those of the MRC, NIHR or the Department of Health and Social Care.

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 PUFFIN trial materials and records for a minimum of 25 years after the close of the trial unless otherwise advised by NCTU or the Sponsor. The Sponsor's trial master file will be archived in accordance with regulatory requirements and local policy.

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 and TSC. Considerations for approving access are documented in the TMG/TSC Terms of Reference.

7.12 Ancillary and Post-trial Care

There are no plans to offer trial treatment to individuals participating in this study after its conclusion. N-3 PUFA is available on prescription should their usual care team wish to prescribe it.

7.13 Publication Policy

7.13.1 Trial Results

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 with any difficulties being resolved by the TSC.

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 TSC approval.

7.14 Patient and Public Involvement

Patient and public involvement representatives will assist the PUFFIN trial team in the design, management and undertaking of the study, and in the dissemination of trial results. Representatives will be consulted during the development of trial documents to ensure they are acceptable to patients with amendments made accordingly.

The PUFFIN trial team will identify and appoint patient and public involvement representatives for each of the TMG and TSC. Their input will be used to guide the undertaking of the research as and where appropriate.

The results of the trial will be discussed with the representatives prior to submission of any formal reports.

8 Ancillary Studies

9 Protocol Amendments

Protocol Version	Date	Summary of Changes
		•

10 References

1. Chan AW, Tetzlaff JM, Altman DG, Laupacis A, Gotzsche PC, Krleza-Jeric K, et al. SPIRIT 2013 statement: defining standard protocol items for clinical trials. Ann Intern Med. 2013;158(3):200-7.
2. Chan AW, Tetzlaff JM, Gotzsche PC, Altman DG, Mann H, Berlin JA, et al. SPIRIT 2013 explanation and elaboration: guidance for protocols of clinical trials. Bmj. 2013;346:e7586.
3. Collaborators GBDCRD. Global, regional, and national deaths, prevalence, disability-adjusted life years, and years lived with disability for chronic obstructive pulmonary disease and asthma, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet Respir Med. 2017;5(9):691-706.
4. Mukherjee M, Stoddart A, Gupta RP, Nwaru BI, Farr A, Heaven M, et al. The epidemiology, healthcare and societal burden and costs of asthma in the UK and its member nations: analyses of standalone and linked national databases. BMC Med. 2016;14(1):113.
5. Shenolikar R, Song X, Anderson JA, Chu BC, Cantrell CR. Costs of asthma among US working adults. Am J Manag Care. 2011;17(6):409-16.
6. Price D, Haughney J, Sims E, Ali M, von Ziegenweidt J, Hillyer EV, et al. Effectiveness of inhaler types for real-world asthma management: retrospective observational study using the GPRD. J Asthma Allergy. 2011;4:37-47.
7. Ehteshami-Afshar S, FitzGerald JM, Doyle-Waters MM, Sadatsafavi M. The global economic burden of asthma and chronic obstructive pulmonary disease. Int J Tuberc Lung Dis. 2016;20(1):11-23.
8. Doz M, Chouaid C, Com-Ruelle L, Calvo E, Brosa M, Robert J, et al. The association between asthma control, health care costs, and quality of life in France and Spain. BMC Pulm Med. 2013;13:15.
9. Bush A, Kleinert S, Pavord ID. The asthmas in 2015 and beyond: a Lancet Commission. The Lancet. 2015:1273-5.
10. Kowalski ML. Diagnosis and management of NSAID-Exacerbated Respiratory Disease (N-ERD)-a EAACI position paper. Allergy. 2019;74(1):28-39.
11. White AA, Stevenson DD. Aspirin-Exacerbated Respiratory Disease. The new england journal of medicine. 2018;379:1060-70.
12. Ledford DK, Wenzel SE, Lockey RF. Aspirin or Other Nonsteroidal Inflammatory Agent Exacerbated Asthma. The Journal of Allergy and Clinical Immunology: In Practice. 2014;2(6):653–7.
13. Rajan JP, Wineinger NE, Stevenson DD, White AA. Prevalence of aspirin-exacerbated respiratory disease among asthmatic patients: A meta-analysis of the literature. J Allergy Clin Immunol. 2015;125(3):676.
14. Szczeklik A, Nizankowska E, Duplaga M. Natural history of aspirin-induced asthma. AIANE Investigators. European Network on Aspirin-Induced Asthma. Eur Respir J. 2000;16(3):432-6.
15. Divekar R, Hagan J, Rank M, Park M, Volcheck G, O'Brien E, et al. Diagnostic Utility of Urinary LTE4 in Asthma, Allergic Rhinitis, Chronic Rhinosinusitis, Nasal Polyps, and Aspirin Sensitivity. J Allergy Clin Immunol Pract. 2016;4(4):665-70.
16. Sastre B, del Pozo V. Role of PGE2 in asthma and nonasthmatic eosinophilic bronchitis. Mediators Inflamm. 2012;2012:645383.
17. Mascia K, Haselkorn T, Deniz YM, Miller DP, Bleecker ER, Borish L, et al. Aspirin sensitivity and severity of asthma: evidence for irreversible airway obstruction in patients with severe or difficult-to-treat asthma. J Allergy Clin Immunol. 2005;116(5):970-5.
18. Laidlaw TM. Clinical updates in aspirin-exacerbated respiratory disease. Allergy and Asthma Proceedings 2019;40(1):4-6.
19. Gabbs M, Leng S, Devassy JG, Monirujjaman M, Aukema HM. Advances in Our Understanding of Oxylipins Derived from Dietary PUFAs. Adv Nutr. 2015;6(5):513-40.

20. Calder PC. Marine omega-3 fatty acids and inflammatory processes: Effects, mechanisms and clinical relevance. Biochim Biophys Acta 2015;1851(4):469-84.
21. Lin N. What is the impact of n-3 PUFAs on inflammation markers in Type 2 diabetic mellitus populations?: a systematic review and meta-analysis of randomized controlled trials. BMC. 2016;20(15):133.
22. Klemens CM. The effect of perinatal omega-3 fatty acid supplementation on inflammatory markers and allergic diseases: a systematic review. BJOG. 2011;118(8):916-25.
23. Broughton KS, Johnson CS, Pace BK, Liebman M, Kleppinger KM. Reduced asthma symptoms with n-3 fatty acid ingestion are related to 5-series leukotriene production. Am J Clin Nutr. 1997;65(4):1011-7.
24. Kumar A, Mastana SS, Lindley MR. n-3 Fatty acids and asthma. Nutr Res Rev. 2016;29(1):1-16.
25. Healy E, Newell L, Howarth P, Friedmann PS. Control of salicylate intolerance with fish oils. Br J Dermatol 2008;159(6):1368-9.
26. Juniper EF, Guyatt GH, Cox FM, Ferrie PJ, King DR. Development and validation of the Mini Asthma Quality of Life Questionnaire. Eur Respir J. 1999;14(1):32-8.
27. EuroQol--a new facility for the measurement of health-related quality of life. The EuroQol Group. Health Policy. 1990;16(3):199-208.
28. Hopkins C, Gillett S, Slack R, Lund VJ, Browne JP. Psychometric validity of the 22-item Sinonasal Outcome Test. Clin Otolaryngol. 2009;34(5):447-54.
29. Wilson AM, Sims EJ, Robb F, Cockburn W, Lipworth BJ. Peak inspiratory flow rate is more sensitive than acoustic rhinometry or rhinomanometry in detecting corticosteroid response with nasal histamine challenge. Rhinology. 2003;41(1):16-20.
30. Oostveen E, MacLeod D, Lorino H, Farre R, Hantos Z, Desager K, et al. The forced oscillation technique in clinical practice: methodology, recommendations and future developments. Eur Respir J. 2003;22(6):1026-41.
31. Pizzichini MM, Popov TA, Efthimiadis A, Hussack P, Evans S, Pizzichini E, et al. Spontaneous and induced sputum to measure indices of airway inflammation in asthma. Am J Respir Crit Care Med. 1996;154(4 Pt 1):866-9.
32. Pizzichini E, Pizzichini MM, Efthimiadis A, Evans S, Morris MM, Squillace D, et al. Indices of airway inflammation in induced sputum: reproducibility and validity of cell and fluid-phase measurements. Am J Respir Crit Care Med. 1996;154(2 Pt 1):308-17.
33. Mulligan AA, Luben RN, Bhaniani A, Parry-Smith DJ, O'Connor L, Khawaja AP, et al. A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. BMJ Open. 2014;4(3):e004503.
34. Barden A, Mas E, Croft KD, Phillips M, Mori TA. Short-term n-3 fatty acid supplementation but not aspirin increases plasma proresolving mediators of inflammation. J Lipid Res. 2014;55(11):2401-7.
35. Comhair SAA, Bochenek G, Baicker-McKee S, Wang Z, Stachura T, Sanak M, et al. The utility of biomarkers in diagnosis of aspirin exacerbated respiratory disease. Respir Res. 2018;19(1):210.
36. Asano K, Lilly CM, O'Donnell WJ, Israel E, Fischer A, Ransil BJ, Drazen JM. Diurnal variation of urinary leukotriene E4 and histamine excretion rates in normal subjects and patients with mild-to-moderate asthma. J Allergy Clin Immunol. 1995 Nov;96(5 Pt 1):643-51.
37. Juniper EF, Svensson K, Mork AC, Stahl E. Measurement properties and interpretation of three shortened versions of the asthma control questionnaire. Respir Med 2005;99(5):553–8.
38. Alzahrani YA, Becker EA. Asthma Control Assessment Tools. Respiratory Care. 2016;61(1):106-16.
39. A D. Sample sizes for clinical trials using sputum eosinophils as a primary outcome. 2013;42.
40. Bochenek G, Stachura T, Szafraniec K, Plutecka H, Sanak M, Nizankowska-Mogilnicka E, et al. Diagnostic Accuracy of Urinary LTE4 Measurement to Predict Aspirin-Exacerbated Respiratory Disease in Patients with Asthma. J Allergy Clin Immunol Pract. 2018;6(2):528-35.

41. Miller B MR, Gane S, Larco J, Sannah AA, Darby Y, Scadding G. Nasal lysine aspirin challenge in the diagnosis of aspirin - exacerbated respiratory disease. 2013;43(8):874-80.
42. Dolan P. Modeling valuations for EuroQol health states. Med Care. 1997;35(11):1095-108.
43. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-43.
44. Wilson A, Dempsey OJ, Sims EJ, Coutie WJ, Paterson MC, Lipworth BJ. Evaluation of treatment response in patients with seasonal allergic rhinitis using domiciliary nasal peak inspiratory flow. Clin Exp Allergy. 2000;30(6):833-8.
45. Sanchez-Jareno M, Barranco P, Padial Vilchez MA, Valbuena T, Lluch M, Dominguez-Ortega J, et al. Changes in Fractional Exhaled Nitric Oxide Levels After Bronchial Challenge With Aspirin in Patients With Aspirin-Induced Asthma. J Investig Allergol Clin Immunol. 2019;29(2):137-9.
46. Schneider TR, Johns CB, Palumbo ML, Murphy KC, Cahill KN, Laidlaw TM. Dietary Fatty Acid Modification for the Treatment of Aspirin-Exacerbated Respiratory Disease: A Prospective Pilot Trial. The Journal of Allergy and Clinical Immunology: In Practice. 2018;6(3):825-31.
47. Harris WS, Thomas RM. Biological variability of blood omega-3 biomarkers. Clin Biochem. 2010;43(3):338-40.
48. Flock MR, Skulas-Ray AC, Harris WS, Etherton TD, Fleming JA, Kris-Etherton PM. Determinants of erythrocyte omega-3 fatty acid content in response to fish oil supplementation: a dose-response randomized controlled trial. J Am Heart Assoc. 2013;2(6):e000513.
49. MHRA HaCRW, NHS Scotland, HSC Health and Social Care, HRA. Joint statement on seeking consent by electronic methods 2018 [Available from: https://www.hra.nhs.uk/about-us/news-updates/hra-and-mhra-publish-joint-statement-seeking-and-documenting-consent-using-electronic-methods-econsent/.
50. Lombardi M, Carbone S, Del Buono MG, Chiabrando JG, Vescovo GM, Camilli M, Montone RA, Vergallo R, Abbate A, Biondi-Zoccai G, Dixon DL, Crea F, Omega-3 fatty acids supplementation and risk of atrial fibrillation: an updated meta-analysis of randomized controlled trials. Eur Heart J Cardiovasc Pharmacother. 2021;7(4):e69-e70.

11 Appendices

12 Principal Investigator compliance statement

All PUFFIN PI's are required to sign a site-specific copy of the investigator compliance statement below prior to activation.

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

PUFFIN

The efficacy and risks of treating people with **asthma** and non-steroidal exacerbated airways disease with **N-3 PUFA** (PUFFIN study): a randomised placebo-controlled multi-centre clinical trial

Trial protocol version	[insert current version number]
Trial protocol date	[insert date of current version]

I, [Insert investigator name], confirm:

1. that [insert name of site] site is willing and able to comply with the requirements of the PUFFIN 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 SPC, 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 [insert name of site] site has 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 PUFFIN 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 PUFFIN 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 Sponsor, 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 PUFFIN trial materials and records for a minimum of 25 years after the close of the trial unless otherwise advised by the NCTU.

Agreement: Principal Investigator

Name	[insert name]
Signature	[insert wet signature]
Date	[insert date]

Please return a copy of this signed agreement (to the NCTU trial team at *PUFFIN@uea.ac.uk)*.