



PARENT-DETERMINED ORAL MONTELUKAST THERAPY FOR PRESCHOOL WHEEZE

(Wheeze And Intermittent Treatment; WAIT)

Study Protocol

Version 1

Signed by Professor Jonathan Grigg
Chief Investigator

Dated: 1st October 2009

Full title of the protocol: Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.

Short title (Acronym): Wheeze And Intermittent Treatment; WAIT

Sponsor: Queen Mary University London
Approval Ref 006963 QM
Representative of the sponsor:
Mr Gerry Leonard (Joint Research Office
Ms Claire Wiltshire (Queen Mary Governance)
5 Walden Street
Whitechapel, London,
E1 2EF
Phone: 0207 882 7260
Email: gerry.leonard@bartsandthelondon.nhs.uk

Funder: **National Institute of Health Research/ Medical Research Council Efficacy and Mechanism Evaluation Programme**
Alpha House, University of Southampton, Science Park,
Southampton,
SO16 7NS,
Tel: 02380594303
Fax: 02380595939

Post Award Manager: **Dr Jane Sinclair**
EME Programme Manager
awards@eme.ac.uk

All Trusts and organisations: **Barts and The London School of Medicine and Dentistry**
Centre for Paediatrics
Institute of Cell and Molecular Science
The Blizzard Building
4 Newark Street, London E1 2AT, UK

Centre for Health Sciences
Institute of Health Sciences Education
2 Newark Street, London E1 2AT, UK

Pragmatic Clinical Trials Unit
2 Newark St, London E1 2AT, UK

Barts and The London NHS Trust

Royal London Hospital
Whitechapel Road
London E1 1BB, UK

University Hospitals of Leicester NHS Trust

UHL Children's Hospital
Leicester Royal Infirmary, Infirmary Square,
Leicester LE1 5WW

University of Aberdeen

Centre of Academic Primary Care
Foresterhill Health Centre
Westburn Road
Aberdeen
AB25 2AY

NHS Grampian Royal Aberdeen Children's Hospital

Department of Child Health
University of Aberdeen
Foresterhill
Aberdeen
AB25 2ZG
Tel; 01224-552471
Fax 01224-551919

Norwich NHS Primary Care Trust

40 Upton Rd
Norwich, NR4 7PA

Norfolk and Norwich University Hospital

Norfolk & Suffolk CLRN, Room 32 (Medical School),
Level 3, East Block, Colney Lane
Norwich
NR4 7UY

List all the Pharmacies:

Pharmacy Department

Royal London Hospital
London E1 1BB

Laboratories:

Institute of Cell and Molecular Science,

Barts and The London School of Medicine and Dentistry,
The Blizzard Building, 4 Newark Street,
Whitechapel,
London E1 2AT, UK

Courier:**City Link Ltd**

Riverbank, Meadows Business Park
Blackwater, Surrey
GU17 9AB, UK

Other Departments:

Not applicable

Date: 1st October 2009

Chief and Principal Investigator:**Professor Jonathan Grigg**

Centre for Paediatrics
Institute of Cell and Molecular Science
The Blizzard Building
4 Newark Street, London E1 2AT, UK
Tel 02078822206
Mobile 07787550774
Fax 02078825555
Email j.grigg@qmul.ac.uk

Clinical Co- Principal Investigators:**Prof Christopher Griffiths**

Professor of Primary Care
Centre for Health Sciences
Barts and The London School of Medicine and Dentistry
2 Newark St, London E1 2AT
Tel 0207 882 2503
Fax 0207 882 2552
Email c.j.griffiths@qmul.ac.uk

Professor David Price

Professor of Primary Care
Centre of Academic Primary Care
University of Aberdeen
Foresterhill Health Centre
Westburn Road
Aberdeen AB25 2AY
Tel : 01224 553066
Fax : 01224 550683
david@respiratoryresearch.org

Also for Professor David Price

Comprehensive Local Research Network Co-director

Norfolk & Suffolk CLRN, Research and Development Office, Room 32 (Medical School),
Level 3, East Block, Norfolk & Norwich University Hospital,
Norwich, NR4 7UY

Dr Stephen Turner

Senior Lecturer in Paediatric Respiratory Medicine
Department of Child Health
Royal Aberdeen Children's Hospital
University of Aberdeen
Foresterhill
Aberdeen
AB25 2ZG
Tel; 01224-552471
Fax 01224-551919
s.w.turner@abdn.ac.uk

Dr Hitesh Pandya

Senior Lecturer in Child Health
Division of Infection Immunity and Inflammation
University Hospitals of Leicester NHS Trust Children's Hospital
University of Leicester
Robert Kilpatrick Clinical Sciences Building
Leicester Royal Infirmary
Leicester, LE2 7LX, UK
Tel 01162525810
Fax 01162522958
hp28@le.ac.uk

Project Manager:

Professor Jonathan Grigg

Centre for Paediatrics
Institute of Cell and Molecular Science
Blizard Building
4 Newark Street, London E1 2AT, UK
Tel 02078822206
Mobile 07787550774
Fax 02078825555
Email j.grigg@qmul.ac.uk

Trial Co-ordinator:

To be appointed

Trial Co-ordinator
Centre for Paediatrics
4 Newark St
London E1 2AT

Tel: 0207 882 2306
Fax: 0207 882 2195
Email b.maclaughlin@qmul.ac.uk

Data Monitor:

Barts and the London Joint Research Office

Supervised by; **Mr Gerry Leonard**

24-26 Walden Street
Whitechapel, London,
E1 2AN

Phone: 0207 882 7260

Email: gerry.leonard@bartsandthelondon.nhs.uk

Study Statistician:

Professor Sandra Eldridge

Professor of Biostatistics

Centre for Health Sciences

Barts and the London School of Medicine and Dentistry

2 Newark St, London E1 2AT

Tel 0207 882 2519

Fax 0207 882 2552

Email s.eldridge@qmul.ac.uk

Data Monitoring Committee members:

Professor Andrew Bush

Professor of Paediatric Respiriology, Imperial College,
Honorary Department of Paediatric Respiratory Medicine,
Royal Brompton Hospital, Sydney Street,
London SW3 6NP, UK.

Tel 020 7352 8121 x2255

E mail a.bush@imperial.ac.uk

Dr Paul Lambert

Reader in Medical Statistics

Centre for Biostatistics & Genetic Epidemiology

Department of Health Sciences

University of Leicester

2nd Floor, Adrian Building

University Road

Leicester LE1 7RH

Tel: 0116 229 7265

Fax: 0116 229 7250

e-mail: paul.lambert@le.ac.uk

Mr Ian Jarrold
Research Manager
British Lung Foundation,
73-75 Goswell Road,
London EC1V 7ER
Tel: 0858050520
E mail Ian.Jarrold@blf-uk.org

Other Study Contacts:

Professor Clive Seale
Professor of Medical Sociology
Centre for Health Sciences
Barts and The London School of Medicine and Dentistry
2 Newark St, London E1 2AT
Tel 0207 882 2503
Fax 0207 882 2552
Email c.seale@qmul.ac.uk

Professor Robert Walton
Professor of Primary Medical Care
Centre for Health Sciences
Barts and The London School of Medicine and Dentistry
Abernethy Building
2 Newark Street
Whitechapel
London E1 2AT
Phone: 020 7882 2502
Fax: 020 7882 2552
r.walton@qmul.ac.uk

Dr Thomas J Vulliamy
Senior Lecturer Molecular Biology
Centre for Paediatrics
Institute of Cell and Molecular Science
The Blizzard Building
4 Newark Street, London E1 2AT, UK
Tel 02078822623
Fax 020278822195
t.vulliamy@qmul.ac.uk

Ms Michelle Moore
Parent Representation Co-ordinator
UHL Children's Hospital
University of Leicester
Robert Kilpatrick Clinical Sciences Building

Leicester Royal Infirmary
Leicester, LE2 7LX, UK
Tel 01162525810
Fax 01162522958
mdl13@le.ac.uk

Dr John Holloway

University of Southampton School of Medicine (Human Genetics Division)
Faculty of Medicine, Health and Life Sciences
University of Southampton
University Road
SO171BJ
Tel 023 8079 8758
Fax 023 8079 6421
j.w.holloway@soton.ac.uk

Dr Hussain Mulla

Co-Director / Researcher in Paediatric Clinical Pharmacology
Centre for Therapeutic Evaluation of Drugs in Children
University Hospitals of Leicester NHS Trust
Glenfield Hospital
Groby Road
Leicester
LE3 9QP
Tel: 0116 2502708
hussain.mulla@uhl-tr.nhs.uk

Table of Contents

Study Summary	13
Glossary of Terms and Abbreviations	14
1 Introduction	15
1.1 Background	16
1.2 Investigational Medical Product	16
1.3 Preclinical data	16
1.4 Clinical data	16
1.5 Rationale and Risk/Benefit Assessment	17
2 Study Aims and Objectives	17
3 Investigational Plan	17
3.1 Overall Design	17
3.2 Schema	17
3.3 Overview of Study Population	19
3.4 Target Accrual	19
4 Subject Selection	19
4.1 Inclusion Criteria	19
4.2 Exclusion Criteria	19
5 Study Procedures and Schedule of Assessments	20
5.1 Informed Consent Procedures	20
5.2 Screening and Registration Procedures	20
5.3 Randomisation procedures	22
5.4 Treatment procedures	22
5.5 Method for assigning subjects to treatment group	22
5.6 Flow diagram	23
5.7 Follow-up procedures	24
5.8 Laboratory assessments	26
5.9 Radiology or other Procedure	26
5.10 Unblinding procedures	27
5.11 Withdrawal	28

6 Investigational Medicinal Products	28
6.1 Definition of each IMP	28
6.2 Product sourcing, manufacture and supply	29
6.3 Pre-medications	29
6.4 Prescription of IMP	29
6.5 Preparation and administration of IMP	29
6.6 Prior and Concomitant Therapies	29
6.7 Dose modification/reduction/delay	29
6.8 Toxicity profiles	30
6.9 Labelling and packaging	30
6.10 Blinding of IMP	30
6.11 Receipt of IMP Supplies and Storage of IMP	30
6.12 Dispensing of IMP	30
6.13 Return and destruction of IMP	30
7 Pharmacovigilance	31
7.1 General Definitions	31
7.2 Investigators' Assessment	32
7.3 Notification and Reporting of Adverse Events and Reactions	32
7.4 Notification and Reporting of Serious Adverse Events / SUSAR	34
7.5 Procedures for reporting Blinded SUSAR	34
7.6 Expected SAEs/SARs and non-reportable events	34
7.7 Pregnancy	35
7.8 Overview of the Safety Reporting Process	35
7.9 Pharmacovigilance responsibilities	35
8 Data Handling and Record Keeping	35
8.1 Confidentiality	35
8.2 Study Documents	35
8.3 Case Report Forms	36
8.4 Record Retention and Archiving	36
8.5 Compliance	36
8.6 Definition of the end of the study	36

9 Clinical Governance Issues	36
9.1 Ethical considerations	36
9.2 Summary monitoring plan	38
9.3 Audit and inspection	38
9.4 Reporting of Serious breaches in GCP or trial protocol	38
9.5 Quality Assurance	38
9.6 Data Monitoring Committee	38
10. Statistics	40
10.1 Endpoints	41
10.2 Statistical considerations	41
10.3 Statistical Analysis	44
10.4 General Considerations	44
10.5 Frequency of Analysis	44
10.6 Analysis of participants' baseline characteristics	44
10.7 Analysis of primary endpoints	44
10.8 Secondary endpoint analysis	44
10.9 Interim Analysis	44
10.10 Randomisation and Stratification	46
11 Study Finances	46
11.1 Funding Source	46
11.2 Subject expenses and payments	46
12 Sponsorship and Indemnity	46
13 Publication policy	46
14 References	48

Study Summary

Full Title	Parent-determined oral montelukast therapy for preschool wheeze with stratification for Arachidonate-5-Lipoxygenase (ALOX5) promoter genotype
Short Title	Wheeze and Intermittent Treatment (WAIT)
Protocol Version Number and Date	Version 1 dated 30 th September 2009
Methodology	Randomised, double-blind, placebo-controlled trial
Study Duration	3 years
Study Centres	Barts and The London NHS Trust; University of Leicester NHS Trust, Norwich Primary Care Trust, Grampian NHS Trust
Primary objective (phase of trial)	To determine whether parent-initiated intermittent treatment with oral montelukast in preschool children reduces the need for unscheduled medical attention for upper or lower respiratory tract infection or wheeze (Phase 3 trial).
Number of Subjects/Patients	1300
Main Inclusion Criteria	<ul style="list-style-type: none"> • Medical record diagnosis of wheeze, two previous attacks of wheeze one being within the last 3 months. • Age \geq 10 months and \leq 5.0 years at recruitment
Statistical Methodology and Analysis	The incident rate ratio (relative risk) and 95% confidence interval for need to unscheduled medical attention.

Glossary of Terms and Abbreviations

AE	Adverse Event
ACT	Asthma Control Test
AR	Adverse Reaction
ALOX5	membrane bound 5-lipoxygenase
ASR	Annual Safety Report
CA	Competent Authority
Child	An individual who takes part in this clinical trial
CI	Chief Investigator
cLT	Cysteinyl Leukotriene
CRF	Case Report Form
CRO	Contract Research Organisation
CTA	Clinical Trial Authorisation
CTIMP	Clinical Trial of Investigational Medicinal Product
DMC	Data Monitoring Committee
EC	European Commission
EMA	European Medicines Agency
EU	European Union
EUCTD	European Clinical Trials Directive
EudraCT	European Clinical Trials Database
EudraVIGILANCE	European database for Pharmacovigilance
GAfREC	Governance Arrangements for NHS Research Ethics
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
IB	Investigator Brochure
ICF	Informed Consent Form
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISRCTN	International Standard Randomised
IVDMIA	<i>In vitro</i> diagnostic multivariate index assay
JRO	Joint Research and Development Office
MA	Marketing Authorisation

MHRA	Medicines and Healthcare products Regulatory Agency
MS	Member State
Main REC	Main Research Ethics Committee
NHS R&D	National Health Service Research & Development
PAG	Parental Advisory Group
PI	Principle Investigator
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person for release of trial drug
RCT	Randomised Control Trial
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SDV	Source Document Verification
SOP	Standard Operating Procedures
SmPC	Summary of Product Characteristics
SSA	Site Specific Assessment
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMG	Trial Management Group
TSC	Trial Steering Committee
URTI	Upper Respiratory Tract Infection

1. Introduction

1.1 Background

A quarter of preschool children between 1 and 5 yrs of age will develop at least one attack of wheeze (1). The majority of affected children have several attacks of wheeze triggered by viral-colds, with minimal or no symptoms between attacks (2). A minority of preschool children will also wheeze between colds. Preschool wheeze is a major clinical problem, with significant costs to primary and secondary care (3) (4). There are at least 2 clinical patterns of preschool wheeze; episodic viral-triggered wheeze which affects the majority of affected children, and multiple trigger wheeze which affects the minority. A promising therapy for both clinical phenotypes of wheeze is montelukast (trade name; Singulair), currently the only cysteinyl leukotriene (cLT) receptor antagonist licensed for young children. This beneficial effect of inhibition of cLT, a class of potent bronchoconstrictors, in preschool wheeze was suggested by our study of urinary cysteinyl leukotrienes, where levels of urinary LTC₄ were elevated during an acute attacks of preschool wheeze, then fell into the normal range on convalescence (5). A study relevant to “multi-trigger” preschool wheeze is a RCT of 689 young children where regular oral montelukast given over a 12 month period reduced the rate of exacerbations by 30% (6). For episodic (viral) preschool wheeze Bisgaard *et al* (7) reported that regular daily use of oral montelukast over 12 months reduced the rate of preschool wheezing episodes by 32% compared with placebo. In a heterogeneous age group of children (that including both preschool and school-age children (n=220), we recruited children aged between 2 and 14 years with intermittent asthma into a 12-month randomised controlled trial of oral montelukast (Pre-Empt study). Trial medication was started at the onset of a viral upper respiratory tract infection and continued for a minimum of 7 days, or until symptoms had resolved for 48 hours (8). 201 children were recruited, a proportion in the preschool age range. The montelukast-treated group had 162 unscheduled health-care resource utilisations for wheeze compared to 288 in the placebo group, and symptoms were significantly reduced by 14% in the montelukast treated group (8). Since intermittent therapy may be effective in preschool wheeze, the aim of this trial is to assess whether parent-initiated montelukast therapy is efficacious in this condition.

The beneficial effect of montelukast, albeit consistent, is clinically relatively “modest” (8). The overall modest benefit of montelukast is due to marked heterogeneity of response; i.e. some children respond very well while others do not respond at all. One explanation for this marked heterogeneity in response is variations in genes coding for components of the LT pathway (9). The first step in LT production is the conversion of LTA₄ by membrane bound 5-lipoxygenase (ALOX5); other names for ALOX5 being 5-LO, and leukotriene A4 synthase) and 5-LO-activating protein (FLAP; encoded by the ALOX5P gene). The regulatory domain of ALOX5 controls leukotriene synthesis by catalyzing the conversion of arachidonic acid to 5(S)-HETE, and further dehydration to the leukotriene A₄. The ALOX5 promoter polymorphism results in a variation in the number of SP1 transcription factor-binding motifs – which alters transcription factor binding, and influences 5-LOX gene expression (10). Five SP1 repeats in the ALOX5 promoter are classified as the “wild” type,

with other numbers of repeats reflecting the "mutant" genotype. Lima *et al* (11) found that adults carrying a variant number of repeats on one allele [x/x or 5/x] (where x is not equal to 5) have a 73% reduction in the risk of having an asthma attack on montelukast compared with homozygotes for the 5-repeat (5/5; wild-type) allele. We therefore hypothesise that overall, parent-initiated montelukast therapy in preschool wheeze will be clinically moderately effective, but that there will be a highly-responsive subgroup of children defined by ALOX5 polymorphism status (i.e. carrying a variant number of repeats on one allele). In this trial we therefore include a stratification step for ALOX5 promoter polymorphism status, to ensure that an equal number of children with the variant and wild type number of SP1 repeats in the ALOX5 promoter receive placebo and active medication.

1.2 Investigational Medicinal Product (IMP)

IMP1 is Montelukast Granules (Merck Sharp and Dohme Limited). It comes as white granules 4 mg per sachet with Mannitol excipient. It is licensed in the UK for the use as add-on therapy in children 6 months to 5 year old patients with "mild to moderate persistent asthma" who are "inadequately controlled on inhaled corticosteroids" (MA number PL 0025/0440). It will be administered by parents over a period of one year. Since we found that overprinting the label is an unsatisfactory method of blinding, the IMP1 will be repackaged using identical packaging material to the licenced medication, but with no manufacturer identification label. Repackaging will be done by Nova Labs (Leicester). Since we are only repackaging, a simplified IMPD dossier will be submitted with the CTA application for the IMP.

The **IMP PL1 placebo** is the excipient Mannitol EP (Pearlitol SD 200). Granules will have the identical morphology and taste to IMP1. It is thus identical to in every respect except for the absence of montelukast. The placebo will be prepared in identical packaging to the active drug by Nova Labs (Leicester)

1.3 Preclinical Data

The cysteinyl leukotrienes are potent inflammatory eicosanoids released from various cells including mast cells and eosinophils. These important pro-wheezing mediators bind to cysteinyl leukotriene receptors (CysLT) found in the human airway and cause airway actions, including bronchoconstriction, mucous secretion, vascular permeability, and eosinophil recruitment. Montelukast is an orally active compound which binds with high affinity and selectivity to the CysLT₁ receptor, and is the only compound in the class which is licensed for use in young children (12)

1.4 Clinical Data

1.41 Laboratory studies

In clinical studies, montelukast inhibits bronchoconstriction due to inhaled leukotriene at doses as low as 5 mg (reviewed in SMPC document (12)).

1.42 Intervention studies

In a 12-week, placebo-controlled study in paediatric patients 2 to 5 years of age, montelukast 4 mg once daily improved parameters of asthma control compared with placebo irrespective of concomitant controller therapy (inhaled/nebulised corticosteroids or inhaled/nebulised sodium cromoglycate). Sixty percent of patients were not on any other controller therapy. Montelukast improved daytime symptoms (including coughing, wheezing, trouble breathing and activity limitation) and night-time symptoms compared with placebo. Montelukast also decreased "as-needed" β -agonist use and corticosteroid rescue for worsening asthma compared with placebo. Patients receiving montelukast had more days without asthma than those receiving placebo. A treatment effect was achieved after the first dose. In a 12-month, placebo-controlled study in paediatric patients 2 to 5 years of age with mild asthma and episodic exacerbations, montelukast 4 mg once daily significantly ($p \leq 0.001$) reduced the yearly rate of asthma exacerbation episodes (EE) compared with placebo (1.60 EE vs. 2.34 EE, respectively), [EE defined as ≥ 3 consecutive days with daytime symptoms requiring β_2 -agonist use, or corticosteroids (oral or inhaled), or hospitalisation for asthma]. The percentage reduction in yearly EE rate was 31.9%, with a 95% CI of 16.9, 44.1.(12).

1.5 Rationale and Risk/Benefit Assessment

1.51 Risks.

Montelukast is licensed for use in preschool children (6 months to 5 years) as a continuous "add on" therapy (12). No child will be denied the main therapy for wheeze in this age group i.e. "as required" inhaled short acting beta₂ agonist (salbutamol). Side effects of montelukast are mild, with a slight excess of headache, ear infection, sore throat, and upper respiratory infection reported in paediatric studies. These side effects have been reported with continuous use and are probably less likely to occur with intermittent therapy. Montelukast can be safely given with all other anti-asthma medications. All children will receive "as required" inhaled salbutamol, and if clinically indicated, may receive regular inhaled corticosteroids. A child may be withdrawn from the trial they experience a serious adverse event which necessitates withdrawal, or if continuous oral montelukast is prescribed.

1.52 Benefits

A recent audit of "asthma" admission in children covering 67 hospitals during the period 1998-2005, found that 75% of 9,429 admissions were for preschool wheeze (3). Since this audit was based in secondary care-based audit, underestimates the total number unscheduled attendances for preschool wheeze. A therapy that reduces the number of severe attacks will therefore have a major benefit to the NHS and children.

2 Study Aims and Objectives

The principal objective of this research is to determine whether intermittent treatment with oral montelukast in preschool children reduces the need for unscheduled medical attention for wheeze. Treatment (IMP) will be initiated by parents or guardians at the onset of every viral upper respiratory tract infection and continued for a minimum of 7 days or until wheeze has resolved for 48 hr, and for every episode of wheeze not triggered by a viral cold, and stopped when symptoms have resolved for 48 hr.

3 Investigational plan

3.1 Overall Design

Randomised, double-blind, placebo-controlled clinical trial

Schema (Key: V = Visit, T = Telephone call, wk = week, m = month, *= in a subgroup of parents)

	V1		V2	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11	T12	V3*
	-2 wk	-1 wk	0 wk	+1 m	+2 m	+3 m	+4 m	+5 m	+6 m	+7 m	+8 m	+9 m	+10 m	+11 m	+12 m	+12 m
Informed consent	X															
Check eligibility criteria	X															
Record baseline demographic and clinical data	X															
Review concomitant medications and need for medical attention	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collect saliva sample for DNA	X															
Qualitative assessment of parental views*			X													X
Height, weight of child	X															
Collect urine sample for urinary leukotriene	X															
Train parents on use of IMP and use of inhaled short-acting beta-2 agonist			X													
Establish ALOX5 polymorphism status		X														
Randomisation		X														
Withdraw IMP from pharmacy and send to local researcher for issue in V2		X							X							
Supply IMP to parents			X		X				X							
Adverse event recording				X	X	X	X	X	X	X	X	X	X	X	X	X

3.3 Overview of Study Population

The study population will comprise preschool children (10 months to 5 years inclusive) with two previous episodes of wheeze.

3.4 Target Accrual

Target accrual is 1300 children.

4 Subject Selection

4.1 Inclusion Criteria

- age \geq 10 months and \leq 5 years on the day of the first dose of IMP.
- two or more attacks of parent-reported wheeze,
- at least one attack with wheeze validated by a clinician
- the most recent attack within the last 3 months.
- contactable by telephone and able to attend one face-to-face review for issue of IMP
- parent or guardian able to give written informed consent for their child participate in the study.

4.2 Exclusion Criteria

- any other chronic respiratory condition diagnosed by a clinician including structural airway abnormality (e.g. floppy larynx) and cystic fibrosis
- any chronic condition that increases vulnerability to respiratory tract infection such as severe developmental delay with feeding difficulty
- history of neonatal chronic lung disease
- current continuous oral montelukast therapy
- in a trial using an IMP in the previous 3 months prior to recruitment.

5 Study Procedures and Schedule of assessments

5.1 Informed consent procedures

5.1.1 The investigator, or a suitably trained person delegated by the investigator (who may be a research nurse or a research assistant who has attended a UK regulations GCP training course) will obtain written informed consent from each parent or guardian prior to participation in this study, following adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study.

5.1.2 At least 24 hr will be given for consideration by the parent or guardian before taking part.

5.1.3 During the consent process, the investigator, or person delegated by the investigator, will explain that parents or guardians are completely free to refuse to enter the study or to withdraw at any time during the study, for any reason.

5.1.4 If new safety information results in significant changes in the risk/benefit assessment for this trial, the consent form will be reviewed and updated if necessary. All children, including those already being treated, will be informed of the new information, provided with a copy of the updated consent form, and asked to give their consent to continue in the study.

5.2 Screening and Registration procedures

5.2.1 Invitation of potential study participants to attend screening visit

A member of the child's GP's usual care team or the hospital paediatric team (as appropriate) will identify potentially eligible children based on age and history of wheeze from reviewing surgery and accident and emergency records. He/she will then approach the child's parents or guardian in person or by posting an invitation letter, to ask if they would like to be contacted about the study by a member of the research team. Individuals who agree to be contacted about the study will then be contacted by a research nurse or research assistant, who will briefly describe the study to them, and ask them if they would like to read a parent information sheet (PIS). The research nurse or research assistant will then post or give a PIS to parents expressing an interest in the study; those who subsequently confirm their interest in participation will be re-contacted and offered a screening appointment at a study site. A second invitation letter will be posted to individuals who do not respond to the first invitation letter.

5.2.2 Preparation for screening visit

Potential participating parents will be offered a screening appointment. They will also be asked to bring their child's usual asthma medication with them when they attend the screening visit.

5.2.3 Screening visit #1 (-2 weeks)

At the screening visit, an investigator, research nurse or research assistant will obtain written informed consent to participate in the trial from parents who are willing to take part in the study. The eligibility of children to participate in the study will then be assessed according to the criteria documented in sections 4.1. and 4.2. The parents of all eligible will be asked to complete baseline assessments of their child's wheeze status including recording of baseline demographic and clinical data and details of concomitant medications, measurement of weight and height, taking a salivary sample using the Oragene paediatric collection system for extraction of DNA and assessment of leukotriene-associated genes, obtaining a urine sample for leukotriene analysis. A follow-up appointment will be arranged for the issue of the IMP.

5.2.4 (-1 week)

DNA will be extracted from the salivary sample in the Institute for Cell and Molecular Science, Barts and the London, and children assigned to either ALOX5 promoter polymorphism "5/5", or "[5/x and x/x]" genotype. Extracted DNA will be stored at -70°C for batch analysis of 50 polymorphisms in 10 genes encoding components of the LT biosynthetic pathway and the LT receptors. The research nurse or research assistant will then assign a randomisation number to that child as per section 5.5 of this protocol, and withdraw the corresponding IMP from pharmacy on behalf of that subject.

5.2.5 Visit #2 (0 months)

The research nurse or research assistant will meet with parents, concomitant medications will be reviewed, and if all baseline data has been collected satisfactorily, issue parents the box containing 50 sachets of the IMP. Children whose parents are willing to participate but who do not meet eligibility criteria (i.e. no wheeze attack within 3 months) at their initial screening visit may be reassessed if they subsequently meet the eligibility criteria at some time in the future. Parents will be taught how to use the IMP, inhaled "as required" salbutamol metered dose inhaler and spacer. The salbutamol inhaler will be weighed.

In a subgroup of 30 families, we will conduct qualitative interviews to establish attitudes towards genetic testing to develop personalised therapy, acceptability of parent-initiated therapy for preschool wheeze, the expected advantages and disadvantages of using the IMP, and their views on consent process and parent information sheet. In these families a further qualitative interview will be done at 12 months at visit 3 (see 4.7) to establish their attitudes towards genetic testing to individualise therapy, acceptability of parent-initiated therapy for preschool wheeze, their experience of using the trial medication, and the difficulties and advantages with parent-initiated therapy. Either one parent will be interviewed or, if they prefer, a joint interview with both parents will be done. Where

possible, interviews will be done at the parental home. Interviewing, transcribing, and analysis of interviews will be done by a research assistant.

5.3 Randomisation procedures

Nova Laboratories Ltd (Leicester) will prepare a total of 2600 boxes of IMP for this trial. Preparation will be done in batches every 6 months (depending on recruitment rate). 1300 boxes will contain 50 sachets each containing montelukast (**IMP1**) and 1300 boxes will contain 50 sachets of placebo. Boxes will be allocated randomisation numbers in blocks of ten using a computer-generated random sequence. Nova Laboratories Ltd will be responsible for generation of the random number sequence and labelling boxes accordingly. Boxes bearing randomisation numbers will be delivered to the pharmacy at Royal London hospital.

5.4 Treatment procedures

IMP will be presented as white granules administered either directly in the child's mouth, or mixed with a spoonful of cold or room temperature soft food (e.g., applesauce, ice cream, carrots and rice). The white sachet containing the granules should not be opened until ready to use. After opening the sachet, the full dose of granules must be administered within 15 minutes. If mixed with food, the granules must not be stored for future use. The granules are not intended to be dissolved in liquid for administration. However, liquids may be taken by the child subsequent to administration. The granules can be administered without regard to the timing of food ingestion. The dose is 1 sachet per day, started when the child has evidence of a viral cold, and stopped after 7 days if no wheeze develops. If a child develops wheeze with a cold the IMP should be continued until 48 hr after the resolution of wheeze. The IMP is also to be started if the child develops wheeze without evidence of a cold and continued for 48 hr after resolution of wheeze. If a child vomits after the administration of the IMP no additional dose is given, and parents record this on the diary chart.

5.5 Method for assigning subjects to treatment group

Randomisation will be stratified according to ALOX5 promoter polymorphisms status. This will yield two groups:

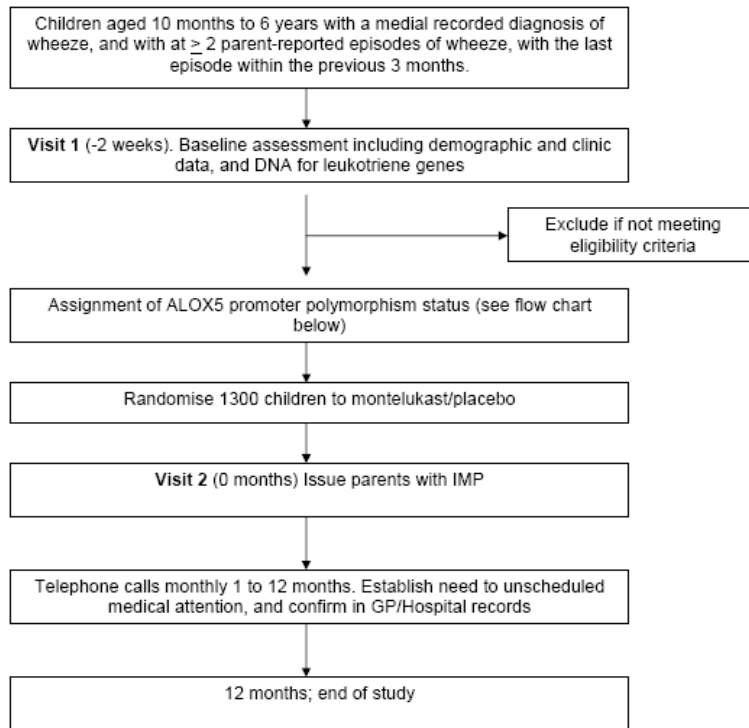
Group I Children with the [5/5] ALOX5 promoter polymorphism genotype.

Group II Children with [5/x and x/x]" ALOX5 promoter polymorphism genotype; where x is > or < than 5 SP1 repeats.

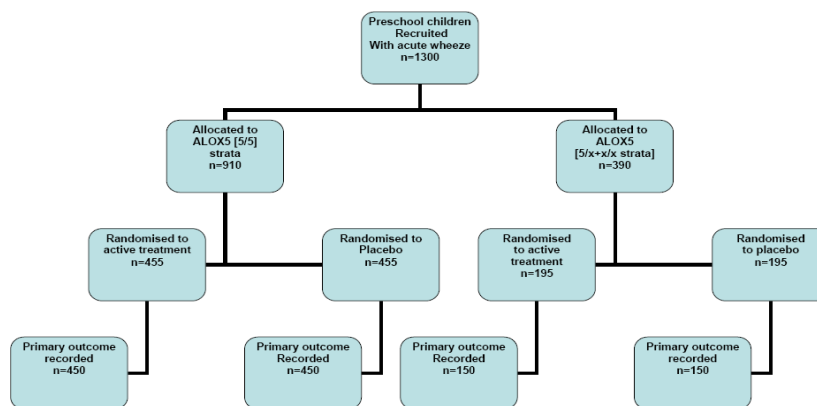
For children (participants) in each of these two genotype groups we will assign consecutive randomisation numbers from randomised permuted blocks of size 12 and 8. Within each block equal numbers of children will be randomly allocated to placebo and active treatment. Block size will be randomly allocated to preserve allocation concealment. When all numbers from the first block (of size 8 or 12) have been assigned a new block (of size 8 or

12) randomisation numbers will be allocated to that group, until a total of 1300 children in groups 1 and 2 combined have been assigned a randomisation number. If a randomisation number is assigned to a child who does not subsequently take any dose of IMP, the IMP bearing that randomisation number will be returned to pharmacy, and the randomisation number may be assigned to another child (participant)

5.6 Flow diagrams



Study design and flow of patients



WAITrial_F

Protocol

Reference 000000000000000000

Date 23rd November 2009

5.7 Follow-up procedures

Parents will be contacted at the following times post-randomisation:

One month: **Telephone call:** a research nurse or research assistant will telephone the parent to ask whether he/she has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Two months: **Telephone call:** a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Three months: **Telephone call:** a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Four months: **Telephone call:** a research nurse or research assistant will telephone the parent to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Five months: **Telephone call:** a research nurse or research assistant will telephone the parent to check whether he/she has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Six months: **Telephone call:** a research nurse or research assistant will telephone the parent to check whether he/she has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so. Parents will be told that

a new IMP box will be posted to them, and on receipt, to return their used IMP box containing unused and used sachets using the freepost envelope provided.

Seven months: Telephone call: a research nurse or research assistant will telephone the parent to check whether he/she has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Eight months: Telephone call: a research nurse or research assistant will telephone the parent to check whether he/she has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Nine months: Telephone call: a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Ten months: Telephone call: a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Eleven months: Telephone call: a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded if so.

Twelve months: Telephone call: a research nurse or research assistant will telephone the subject to check whether the parent has initiated the IMP, the numbers of days the IMP was used, use of healthcare resources, concomitant medications, adverse events, procedures, days lost from childcare, and parent days lost from work. Parents will also be asked if their child experienced any adverse events, and these will be recorded. Parents will be asked to return their used IMP box containing unused and used sachets. Their salbutamol inhaler will be weighed. Parents will be asked to comment on their view on the efficacy of the IMP.

Twelve months: Visit 3 in a subgroup of 30 families: A research nurse or research assistant will visit the home of 30 families originally interviewed for their views in visit 2 to establish their attitudes towards genetic testing in order to individualise therapy,

acceptability of parent-initiated therapy for preschool wheeze, their experience of using the trial medication, and any difficulties/advantages of the parent-initiated approach

Parents will contact the research nurse or research assistant at the following times post-randomisation:

Parents will be asked to contact the research nurse when they initiate via a freephone number. Data recorded in a follow up telephone call from the research nurse include the number of days of wheeze, GP attendance, admission to hospital, need for additional asthma therapy, adverse events, procedures, days lost from childcare, and parent days lost from work. If a parent mislays the IMP supplied, the CI will contact Nova laboratories with details of that child's randomisation number, and ask them to supply a replacement box of IMP containing active or placebo medication according to that subject's randomisation number.

5.8 Laboratory Assessments

Laboratory assessments of genetic polymorphisms and urinary leukotriene are specified in section 10 of this protocol will be performed according to approved standard operating procedures (SOPs).

5.9 Radiology or other Procedure

No radiological examination will be performed for study purposes. Other procedures to be performed are:

Weight: Weight in light clothing will be measured with weighing scales and recorded in kilograms

Salivary sample: Saliva for DNA will be collected using the Oragene-infant sponge system. The sponge tips are cut into an Oragene DNA kit to preserve the DNA and prevent bacterial growth. This method yields high-quality DNA and eliminates the need for traditional cheek scraping method.

ALOX5 polymorphism status will be determined within 1 week of sampling in the ICMS laboratory. DNA is extracted according to a SOP and the manufacturer's instructions (DNAgenotek). Products of the PCR are analysed by capillary electrophoresis on a 3130xl Genetic Analyser (Applied Biosystems). Fragments are obtained, varying in size depending on the copy number of the repeat sequence, and are visualized using GeneMapper v4 software. Genotypes will be called from duplicate amplifications with respect to standards run on each plate that are verified by direct DNA sequence analysis. Within 24 months of collection we will assess 150 polymorphisms in 10 genes encoding components of the LT biosynthetic pathway and the LT

receptors ALOX5, ALOX5AP, LTC4S, CYSLTR1, CYSLTR2, PLA2G4A, LTA4H, LTB4R1, LTB4R2 and MRP. These included all SNPs located in promoter regions, exons and intron-exon boundaries and the SNPs within the ALOX5AP haplotypes (referred to as Hap A and Hap B). Additional tagSNPs will be selected using the LDselect algorithm on the basis of linkage disequilibrium patterns across the genes using data from both our own previous studies in cardiovascular disease and asthma as well as resequencing data available from the Seattle SNPs and NIEHS SNPs databases. SNP genotyping will be carried out using the KASPar competitive allele-specific PCR method (Kbiosciences, Hitchin, UK).

Urine sample A urine sample will be obtained from children in spontaneously voided urine using a sterile cardboard potty. Urinary leukotriene will be assessed using a commercial ELISA kit in the ICMS within 12 months of collection.

5.10 Unblinding procedures

At the time of generating the randomisation code described in 5.3, Nova Laboratories Ltd will also prepare 2 opaque envelopes, each containing a copy of the randomisation code detailing the allocation associated with each randomisation number. The trial pharmacist and a member of the Data Monitoring Committee will hold a copy of the randomisation code.

These envelopes may only be opened in the following circumstances:

1. Emergency code break

A Principal Investigator, or physician covering for that Principal Investigator in his / her absence, may request an emergency code break in the following circumstances:

- a) in case of a suspected severe adverse reaction (SAR) where knowledge of patient allocation may influence clinical management of a study participant
- b) in case of a suspected unexpected severe adverse reaction (SUSAR)
- c) in any other circumstance in which the PI considers that an emergency code-break is indicated

2. Non-emergency code break, which will occur at the end of the trial, to allow data analysis.

5.11 Withdrawal

5.11.1 Withdrawal of Subjects

Children will be withdrawn from the study if their parents withdraw consent to participate, if their allocation is unblinded, if an investigator concludes that this course of action is in the child's best interests. Children will also be considered to have withdrawn if their parents consistently fail to respond to telephone calls at scheduled telephonic review.

5.11.2 When and How to Withdraw Subjects

Children may withdraw from the trial, or be withdrawn from the trial, in the circumstances described in section 5.11.1. If a child or is withdrawn, from the trial, the reason for withdrawal will be recorded in the case report form. When a parent fails to respond to telephone calls at scheduled telephonic review, research nurses or research assistants will make appropriate attempts to contact the parent.

5.11.3 Data Collection and Follow-up for Withdrawn Subjects

Parents of all children who withdraw prematurely from the study will be asked if they agree for continuing telephone calls as detailed in the schedule above. Data collected will be included in study analyses.

6 Investigational medicinal products

6.1 Definition of each IMP

6.1.2 Active drug

Trade name:	Singulair Granules
Composition:	4mg Montelukast sodium (which is equivalent to 4mg montelukast) granules with mannitol expient
ATC code:	R03DC03
Pharmaceutical form:	Granules
Dosage regimen:	One sachet to be given once a day at the start of a cold, and continued for 7 days if no wheeze occurs or until wheeze has resolved for 48 hr, and if wheeze occurs in the absence of a cold to be continued until wheeze has resolved for 48 hr.
Route of administration:	Oral
Manufacturer	Merck Sharpe and Dohme Ltd

6.1.2 Placebo

Trade name:	Mannitol EP (Pearlitol SD 200)
Composition:	Mannitol Granules

ATC code: Not applicable; drug master file lodged with the European Pharmacopoeia commission
Pharmaceutical form: Granules
Dosage regimen: One sachet to be given once a day at the start of a cold, and continued for 7 days if no wheeze occurs or until wheeze has resolved for 48 hr, and if wheeze occurs in the absence of a cold to be continued until wheeze has resolved for 48 hr.
Route of administration: Oral
Manufacturer: Roquette Pharma

6.2 Product sourcing, manufacture and supply

Authorisation number: MIA(IMP) 13581

Authorisation holder: Nova Laboratories Ltd., Martin House, Gloucester Crescent, Wigston, Leicestershire, LE18 4YL

6.3 Pre-medications

Not applicable.

6.4 Prescription of IMP

Trial delegation logs will specify the names of physicians authorised to prescribe IMP for this study.

6.5 Preparation and Administration of IMP

IMP will be prepared by Nova Laboratories Ltd, and shipped to The Royal London Hospital pharmacy. Administration of IMP to be given by the parent or guardian at home at the onset of every viral upper respiratory tract infection and continued for a minimum of 7 days or until symptoms have resolved for 48 hr, and for every episode of wheeze not triggered by a viral cold, and stopped when symptoms have resolved for 48 hr. Parents will be advised to store the IMP at room temperature, out of the reach of children, and instructed on when to initiate and stop treatment

6.6 Prior and Concomitant Therapies

Prohibited concomitant therapies are:

Montelukast granules
Treatment with another IMP

6.7 Dose modification/reduction/ delay

Administration of IMP will be discontinued in the following circumstances:

1. If the child is prematurely withdrawn from the study if their parents withdraw consent to participate.
2. If an investigator concludes that this course of action is in the child's best interests.
3. If a child is prescribed regular daily montelukast by a clinician.

Children who discontinue study drug for any reason other than those specified in (1.) should not be considered to have withdrawn from the study, and their parents should continue to be contacted by telephone.

6.8 Toxicity profiles

The toxicity profile of montelukast granules is minimal and is described in the SPC (12). This will be reviewed annually and updated if relevant new data come to light.

6.9 Labelling and Packaging

IMP will be labelled and packaged by Nova Laboratories Ltd. according to principles of GMP. IMP will be packaged into white sachets.

6.10 Blinding of IMP

All the following individuals will be blinded to the IMP, the parent, the child, the research nurse, the local and chief PIs. Active and placebo batches of IMP will have identical packaging, labelling and appearance.

6.11 Receipt of IMP Supplies and Storage of IMP

The Royal London Hospital pharmacy will inform the Chief Investigator on receipt of IMP deliveries. IMP will be stored in the pharmacy according to GCP principles.

6.12 Dispensing of IMP

Research nurses or research assistants will withdraw IMP from The Royal London Hospital pharmacy on behalf of study participants on production of a signed trial prescription. Parents will be supplied with their first dose of IMP sachets at visit 2; and reissued with a new box of IMP sachets at 6 months.

6.13 Return and Destruction of IMP

Research nurses or research assistants will return unused or expired IMP to the Royal London hospital pharmacy, where it will be destroyed according to local standard operating procedures.

7 Pharmacovigilance

7.1 General definitions

7.1.1 Adverse Events (AE)

An AE is any untoward medical occurrence in a subject (in this case “child”) to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporarily associated with the use of an IMP, whether or not it is considered related to the IMP.

7.1.2 Adverse Reaction (AR)

An AR is any untoward and unintended response in a child to an IMP, which is related to any dose administered to that subject. All adverse events judged by either the reporting investigator or the Sponsor as having a reasonable causal relationship to a medicinal product qualify as adverse reactions. The expression reasonable causal relationship means to convey in general that there is evidence or argument to suggest a causal relationship.

7.1.3 Serious Adverse event (SAE) or Serious Adverse Reaction (SAR)

Serious Adverse Event (SAE)

A SAE fulfils at least one of the following criteria:

Is fatal – results in death (NOTE: death is an outcome, not an event)

Is life-threatening

Requires inpatient hospitalisation or prolongation of existing hospitalisation

Results in persistent or significant disability/incapacity

7.1.4 Suspected Serious Adverse Reaction (SSAR)

An SSAR is an adverse reaction that is classed as serious and which is consistent with the information about the medicinal product as set out in the Summary of Product Characteristics (SmPC) or Investigator’s Brochure (IB) for that product.

7.1.5 Suspected unexpected Serious Adverse Reaction (SUSAR)

The definition of a SUSAR is any suspected unexpected adverse reaction related to an IMP that is both unexpected and serious. In this case the event is not outlined in the Summary of Product Characteristics (SmPC) or Investigator's Brochure (IB) for that product.

7.2 Investigators' Assessment

7.2.1 Seriousness

Responsibility for evaluation of seriousness of adverse events will be shared between the treating principal investigator and the chief investigator, according to criteria specified above.

7.2.2 Causality

Responsibility for evaluation of causality of adverse events will be shared between the treating the chief investigator and clinical co-principal investigators.

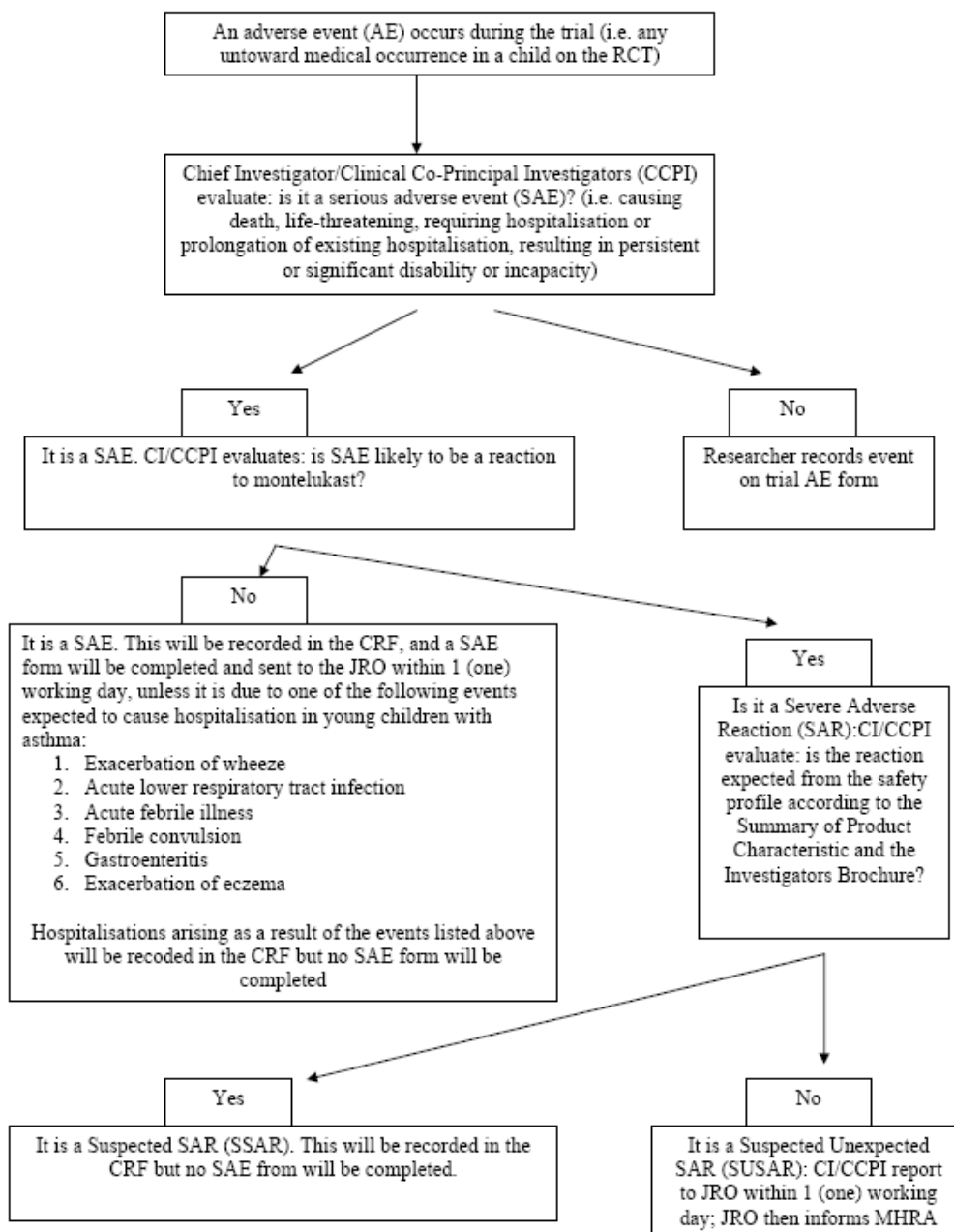
7.2.3 Expectedness

Responsibility for evaluation of expectedness of adverse events will be shared between the treating clinical co-principal investigators and chief investigator.

7.3 Notification and Reporting of Adverse Events or Reactions

Adverse events or reactions will be notified and reported according to the flowchart below.

7.3.1 Flowchart summarising procedures for notification and reporting of adverse events



7.4 Notification and Reporting of Serious Adverse Events / SUSAR

7.4.1 As the IMPs used in this project are licensed in the UK, the expected SARs (outlined in the SmPC) will be recorded in the CRF. SAE forms for these SARs will not be sent to the sponsor. In addition, the population of children entered on this trial (children with at least 2 episodes of wheeze aged 10 months to 5 years inclusive at recruitment) will be expected to be hospitalised as part of their clinical care for the following reasons:

1. Exacerbation of wheeze
2. Acute lower respiratory tract infection
3. Acute febrile illness
4. Febrile convulsion
5. Gastroenteritis
6. Exacerbation of eczema

The SAEs above will be recorded in the CRF, but they will not be recorded on SAE forms or reported to the sponsor as they are classified as expected.

7.4.2 Unexpected SAEs will be recorded in the CRF and on the sponsor SAE form and reported to the JRO within one working day of the PI or co-investigators becoming aware of the event. The co-investigators listed in this protocol will be authorized to sign the SAE forms in the absence of the PI. SUSARs arising during the trial will be reported to the JRO and the main REC within one working day of the PI or co-investigator becoming aware of the event.

7.4.3 Annual Safety Reports (ASR) will be sent by the CI to the sponsor, the MREC and the MHRA on the anniversary of the date on the “notice of acceptance letter” from the MHRA using the sponsor ASR form. The ASR will state whether new findings for the subjects of the trial have changed the benefit / risk ratio of conducting the trial.

7.4.4 Annual Progress Reports will be sent to the main REC and to the sponsor on the anniversary date on the MREC “favourable opinion” letter.

7.5 Procedures for reporting Blinded SUSAR

The allocation of any study participant experiencing a suspected SUSAR will be unblinded according to the WAIT Trial Standard Operating Procedure for emergency code breaking, and reported to the Sponsor. If allocation is to montelukast granules, the sponsor will report the SUSAR to the MHRA.

7.6 Expected SAEs/ SARs and non-reportable events

Expected SAEs / SARs and non-reportable events will be recorded in children’s case report forms.

7.7. Pregnancy

Not applicable (preschool children only).

7.8 Overview of the Safety Reporting Process

An overview of the safety reporting process is presented in section 7.3 of this protocol.

7.9 Pharmacovigilance responsibilities

Pharmacovigilance responsibilities will be shared by the Sponsor and by the Chief Investigator and co-investigators named on this protocol.

8 Data Handling and Record Keeping

8.1 Confidentiality

Children's personal data will remain confidential, and will be handled, processed, stored and destroyed according to the terms of the Data Protection Act 1998.

8.2 Study Documents

Study documentation will be maintained in a Trial Master File, to be stored at the Centre for Paediatrics, Barts and The London Medical School.

8.3 Case Report Forms

Case Report Forms will include the following data: children's demographic details, medical and surgical history, concomitant medications, checklist of eligibility criteria, results of respiratory function tests, results of point-of-care tests, and records of administration of IMP.

8.4 Record Retention and Archiving

Trial records will be retained for 20 years. Records will be stored in the Centre for Paediatrics, Barts and The London Medical School while the study is being conducted, after which they will be transferred to the Barts and the London Trust archive.

8.5 Compliance

The CI & PI will ensure that this study is conducted in accordance with the latest version of the "Declaration of Helsinki" (<http://www.wma.net/e/policy/b3.htm>), the UK Legal Framework for Clinical Trials of Investigational Medical Products (SI 2004/1031) and subsequent relevant amendments. The study will adhere to the principles outlined in the Guidelines for Good Clinical Practice.

8.6 Definition of the end of the study

The end of the study will be defined as the date of the final study visit of the final participant undergoing follow-up.

9 Clinical governance issues

9.1 Ethical considerations

The main ethical considerations arising from the design and conduct of this trial are as follows:

a) Rationale for research

Ethical research must be informed by existing research, and investigate an important question. We have addressed this issue by thorough review of the existing literature, and our own data showing increased leukotriene activation in preschool wheeze (5). There is a consensus among investigators that clinical trials of parent-initiated montelukast are needed to determine whether montelukast can reduce the risk of a severe exacerbation of wheeze leading to need unscheduled medical attention.

b) Design of research

Ethical research must employ the most appropriate design in order to answer the research question. When investigating efficacy of a clinical intervention, a double blind, randomised, placebo-controlled trial is the gold standard methodology.

c) Minimisation of inconvenience, discomfort and risk for participants

Ethical research must seek to minimise potential inconvenience, discomfort and risk that children and their parents may experience during the course of a study. The principle inconveniences of the study arise from the time spent by parents to attend for the screening visit and Visit 2 where the IMP will be issued. We have sought to minimize these by providing reimbursement of reasonable travel expenses incurred as a result of participation in the study. The principle discomfort involved arises from collecting samples for DNA analysis. We have sought to minimize this by using a specially designed saliva collection system for infants (Oragene-infant DNA collection system) which does not need scraping of the buccal mucosa. The risks associated with montelukast therapy are very low, with no significant risks reported. We have sought to minimise this further by review by the data monitoring committee of accumulating data relating to adverse events.

d) Recruitment procedure

Ethical research projects should seek to recruit children from all ethnic backgrounds, and not be restricted to those fluent in the English language. To reduce misunderstandings and to maximise recruitment and adherence of ethnic minorities, the research nurse in London will be expected to understand speak Sylheti and translations of the information sheet will be available in Bengali and Turkish (the main minority languages in Leicester and East London) at all sites.

e) Confidentiality

Ethical research projects should ensure that participants' personal data remain confidential. Our procedures for handling, processing, storage and destruction of data are compliant with the Data Protection Act 1998.

f) Data Handling and Record Keeping

Case report forms will be anonymised and held in locked filing cabinets in the local recruitment centres and in the Centre for Paediatrics, Paediatric Pragmatic Clinical Trial Section, Barts and The London School of Medicine and Dentistry. Only trial staff will have access to these.

Trial staff will enter all non-patient identifiable data from case report forms into an electronic database held on a password protected computer, linked to an ID code which will be unique for each trial participant. One electronic copy of this database will subsequently be stored on a password-protected file on the Chief Investigator's password-protected G-drive

on the QMUL network; a back-up copy will be held on a password-protected file on the C drive of a stand-alone desk-top computer kept in a locked office in the Centre for Health Sciences, Barts and The London School of Medicine and Dentistry. Trial staff and representatives of the sponsor, participating NHS Trusts or regulatory authorities will be the only people with potential access to view study data that could be linked to patient identifiable data.

9.2 Summary monitoring plan

The CI will ensure that the Barts and the London monitoring template is completed in a continuous fashion throughout the study and kept up to date by the CI/co-investigators (for the first part of the report) and by the monitor to be appointed (for both the first part and the source data verification part of the template). This trial uses IMPs licensed in the UK and used within their marketing authorisation. The monitoring report will be sent to the JRO six months after the first consent form has been signed and bi-annually thereafter. The sponsor will carry out random on-site monitoring checks to ensure that the reports have been completed accurately.

9.3 Audit and inspection

Trial documentation will be made available to auditors and inspectors representing the Sponsor and the regulatory authorities.

9.4 Reporting of Serious breaches in GCP or trial protocol

Serious breaches in GCP and serious breaches of the trial protocol will be reported to the sponsor.

9.5 Quality Assurance

Data quality will be audited according to GCP guidelines, and a trail will be maintained of any change or correction to the case report form or the electronic database. The sponsor will have direct access to all trial-related sites, source data and reports in order to ensure that the trial is conducted, and data are generated, recorded and reported in compliance with the protocol and with Good Clinical Practice.

9.6 Data Monitoring Committee

A data monitoring committee (DMC) will be established for the trial. The chairman of the DMC will keep a record of DMC communications and activities. The central responsibilities of this DMC will be to:

a) Maintain trial treatment randomisation codes and unblind individual children's allocation in the circumstances specified in this protocol

b) Make recommendations to the Sponsor and the Chief Investigator on further conduct of this trial, based on results of the monitoring procedures described below. Such recommendations could include continuing or terminating the trial, or modifying its protocol. Any such modifications should not violate the concepts behind the original study protocol. If changes in the study conduct are recommended by this DMC, sufficient information should be provided to allow the sponsor and chief investigator to decide whether and how to implement them. The implementation of any DMC recommendation is the responsibility of the sponsor and chief investigator who are also free to neglect (in whole or in part) any recommendations of this DMC. The sponsor and the investigators bear the final responsibility for the conduct of the trial. This responsibility cannot be transferred to the DMC.

9.6.1 Maintenance of trial treatment randomisation codes and procedures for breaking codes.

The trial treatment randomisation code will be generated by Nova Laboratories Ltd as described above. Nova Laboratories Ltd will email a copy of this code to each member of the DMC, and to the Trials Pharmacist at the Royal London Hospital Pharmacy. A DMC member or a member of the Royal London Hospital Pharmacy staff may unblind a participant's allocation at the request of a Principal Investigator, according to the WAIT Trial Standard Operating Procedure for emergency code breaking.

9.6.2 Monitoring procedures

The DMC will review accumulating data in a blinded fashion in order to monitor safety, efficacy and quality of study conduct. The DMC may also request data presented in an unblinded form.

a) Safety monitoring

Every 6 months, the chief investigator will collate and clean data detailing all fatal / life-threatening events and other serious adverse events to date in trial participants, and email this data to DMC members. The DMC may ask for an analysis to compare the incidence of fatal/life-threatening events and other SAE between the 2 groups, and inform the chief investigator whether new findings for the subjects of the trial have changed the benefit / risk ratio of conducting the trial.

b) Efficacy monitoring

No interim efficacy analysis will be done.

c) Monitoring quality of study conduct

If the DMC observes problems with the study conduct (e.g. with respect to protocol adherence or withdrawal of children), it should consider making recommendations to the investigators and the sponsor to improve the quality of the study.

9.6.3 Declaration of possible conflicts of interest of DMC members

The members of this DMC have no involvements that might raise the question of bias in their reports to the sponsor or investigators in this study. Specifically, they have no financial interest in the outcome of this study, and they will not be authors on publications arising from this study.

9.6.4 Frequency and format of DMC meetings

It is anticipated that the DMC will be able to conduct all of its business by email and telephone call, rendering a physical meeting between members, investigators and representatives of the sponsor unnecessary. The DMC will decide the frequency of the safety analysis.

9.6.5 Communication Procedures

If the DMC chooses to perform a safety analysis, it will communicate results of safety and efficacy analyses by email to the chief investigator.

9.6.6 Responsibilities, timelines and methodological aspects

Members of the DMC will be responsible for conducting safety analyses. It is anticipated that the results of these analyses will be available to the chief investigator within two weeks of submitting data to the DMC. Safety analysis will be performed by comparison of proportion of participants in the 2 arms experiencing severe adverse events using a χ^2 test. There will be no interim efficacy analysis.

9.6.7 Documentation of the DMC activities

The DMC will provide the CI with details of any safety and efficacy analyses performed. The DMC will also keep a record of the circumstances in which it unblinded a participant's allocation.

9.6.8 Parental Advisory Group (PAG)

Each Centre will convene a parental advisory group consisting of at least 4 families with a child who has or had preschool wheeze and who was not recruited in the trial. Parents will guide researchers on how to approach families, will comment on the results of the qualitative interviews, and advise on how best to disseminate the trial's results. The PAG will be chaired by the local PI and will be co-ordinated by Ms Michelle Moore.

10 Statistics

10.1 Endpoints

10.1.1 Primary endpoints

The primary outcome measures for this trial is;

- Number of times a child attends for an unscheduled medical opinion with wheeze over a 12 month period.

10.1.2 Secondary Endpoints

Respiratory morbidity

- Number of days with parent-reported wheeze over the 12 month trial period
- Number of admissions to hospital over the 12 month trial period
- Duration of admissions to hospital over the 12 month trial period
- Time to first attack of wheeze
- Number of unscheduled GP consultations for wheeze
- Duration of episodes by diary card
- Severity of episodes by diary card
- Parent's overall impression of efficacy of IMP

Health service use

- Unscheduled GP consultation with exacerbation of wheeze, expressed as time from randomisation to first attendance and annual attendance rate
- A&E attendance with wheeze exacerbation, expressed as time from randomisation to first attendance and annual attendance rate
- Unscheduled hospital admission with wheeze exacerbation, expressed as time from randomisation to first admission and annual rate of admissions
- Total duration of hospital admissions for exacerbation of wheeze

Adverse events

- Severe adverse events
- Withdrawal from the trial
- Mortality due to exacerbation of asthma
- Mortality due to respiratory infection
- All-cause mortality

Medication use

- Use of oral corticosteroids, expressed as number of courses taken per year, and proportion of children receiving at least one course of oral corticosteroids during the trial
- Use of inhaled relief medication (salbutamol), expressed as total number of occasions used over 12 month period, and mean number per wheeze episode
- Use of inhaled corticosteroids (ICS), expressed as mean daily dose of beclometasone equivalent over the 12 month trial period
- Regular prescription of inhaled ICS over the 12 month trial period

Inflammatory outcomes

- Association between baseline urinary cysteinyl leukotriene level and:
 - ALOX5 status
 - Other polymorphisms of leukotriene genes
 - Previous history of viral-triggered episodic and multi-trigger
 - Responsiveness to montelukast

Genetic parameters

- Differential responsiveness to montelukast for the primary outcome in the stratum with ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x + x/x]” genotype.
- Differential responsiveness to montelukast for the primary outcome resulting from other polymorphisms in genes influencing leukotriene synthesis, leukotriene metabolism and leukotriene activity.

Economic outcomes

- Costs incurred by parents due to wheeze episode (including costs of travel to health care facility, childcare, and days absence from work)
- Costs of medical care provided for exacerbation of wheeze

Qualitative outcomes (parental)

- Attitudes towards genetic testing in order to personalise therapy
- Acceptability of parent-initiated therapy for preschool wheeze
- Experience of using the trial medication
- Difficulties/advantages of the parent-initiated approach
- Views on parent information sheet

10.1.3 Study definitions

1. Need for unscheduled medical attention will be defined as an episode requiring an unscheduled attendance to either a general practitioner, or to an accident and emergency department, or a combination of both - where wheeze is diagnosed by a clinician.

3. Time from randomisation to first attack of severe wheeze will be defined as the number of days from the date of administration of first dose of IMP to the first date on which a wheeze exacerbation attains criteria of severity stated above.

4. Number of days with parent-reported wheeze will be defined as the number of days with wheeze over the 12 month trial period obtained by telephone contact with the researcher.

5. Use of reliever medication will be defined as the number of occasions on which reliever medication is used during the 12 month trial period.

6. Use of inhaled relief medication, expressed as total number of occasions used over 12 month period, and mean number per wheeze episode will be defined from the number of actuations calculated by change in inhaler weight,

10.2 Statistical considerations

10.2.1 Sample Size

This trial is powered to detect a clinically significant difference in the number of attacks of wheeze between intervention and control arms. We also have some power to detect differential responsiveness (in terms of the primary outcome) to montelukast in the stratum with ALOX5 promoter polymorphism [5/5], compared with the stratum with the ALOX5 [5/x and x/x]” genotype.

Baseline data on mean (0.76) and standard deviation (1.22) of number of attacks are based on data from the UK General Practitioner Research Database on courses of oral steroids (a proxy for number of episodes). These data follow an overdispersed poisson distribution. To take account of this we used markov chain Monte Carlo simulation in WinBUGs to estimate sample sizes required: (WinBUGS Version 1.4. 2003 Available from: <http://www.mrc-bsu.cam.ac.uk/bugs/welcome.shtml>). To detect a 33% drop in attack rate requiring medical attention, with a power of 90% and at a significance level of 5%, and a 6% loss to follow up, we require 1050 children in total. A 33% drop in attack rates equates to an attack rate of 0.51 for the treatment group. The clinical significance of these changes is that approximately four children will need to be treated to prevent one clinically severe attack. A sample size of 1200 gives just over 80% power at the 5% significance level to detect an interaction between treatment and genotype if the effect is a 60% reduction in the [5/x plus x/x] and a 20% reduction in the [5/5] stratum. Assuming a 6% dropout, 1300 children will need to be recruited.

10.2.2 Planned recruitment rate

We plan to recruit 1300 children (participants) over 3 years.

10.3 Statistical Analysis

Poisson regression analyses will be applied to determine influence of allocation on the primary outcome and any differential response between the two strata as described below.

10.4 General considerations

Statistical analysis will be performed by a suitably qualified statistician under the supervision of Professor Eldridge.

10.5 Frequency of Analysis

Safety analyses will be conducted at 6-monthly intervals. Efficacy analyses will be conducted on termination of the trial.

10.6 Analysis of children's baseline characteristics

Following data entry and data cleaning, baseline characteristics including age, sex, ethnic group, ongoing asthma therapy will be compared between intervention and control groups.

10.7 Analysis of primary endpoints

Initial analyses will be performed according to intention-to-treat for all participants with outcome data. Per protocol efficacy analyses will also be performed, excluding data collected after discontinuation of IMP for those participants who discontinue IMP. We will use poisson regression with a random effect representing individuals to account for overdispersion. Fixed effects will represent the stratification factor (ALOX5 promoter) and treatment centre. The incident rate ratio (relative risk) and 95% confidence interval will be calculated. Analysis will be conducted in Stata version 10. To test for a differential effect by strata an interaction between strata and treatment will be fitted to this model as described in 10.8.1.

10.8 Secondary endpoint analysis

A Poisson regression analysis with a random effect for individuals to allow for overdispersion will be applied to determine the influence of allocation on number of days with parent-reported wheeze, number of admissions to hospital, number of admissions to hospital > 4 hrs duration. An incident rate ratio for each factor will be presented with 95% confidence intervals.

Time to first attack of wheeze will be analysed using a log-rank test with adjustment for clustering and, if hazards are proportional, Cox's proportional hazards models adjusting for clustering. In a Cox model, strata and centre will be included as covariates.

Other continuous variables will be analysed with analysis of covariance. Dichotomous variables will be analysed with logistic regression analysis.

Adverse events will be analysed with descriptive statistics.

10.8.1 Genetic Analysis

To assess the difference in responsiveness to montelukast in the two ALOX5 strata we will fit an interaction term to test for the interaction between montelukast and stratum in our main model, or each treatment limb. We will also report the associations between genotype and clinical phenotype, urinary leukotriene level, and clinical outcome. To test the polymorphisms in each gene in combination, we will use a composite likelihood approach which combines the regression coefficients for all polymorphisms at each locus. Analysis of clinical effectiveness (utility) of stratification of ALOX5 status will utilise *in vitro* diagnostic multivariate index assays (IVDMIA's). We will estimate the benefits of a multivariate index assay based on our data in both clinical and economic terms (e.g. days off school days off work for parents' costs of attendance at GP and hospital, costs of treatment).

10.8.2 Pre-specified sub-group analyses

There is no pre-specified subgroup analysis, other than the effect of ALOX5 genotype detailed above.

10.8.3 Health economic analysis

Determination of costs

Costs will be obtained by recording units of resources used, and applying tariffs to each. Important units are visits to primary care, use of out-of-hours services and A&E, prescriptions, over-the-counter drugs, hospitalisations and bed-days. Resource use will be costed using the appropriate national tariff for each type of unit cost; when unavailable, we will use the costs charged for tests carried out in the study. Unit costs for general practitioner and nurse consultations, other primary care services and outpatient attendances will be obtained from the Unit Costs of Health and Social Care 2005. These unit costs are inclusive of ancillary staff costs, overheads and training costs. Unit costs for elective and acute hospital admissions will be obtained from the Reference Costs Database. Unit drug costs per daily dose will be calculated from the Prescription Cost Analysis Database. Unit costs for diagnostic tests will be obtained from published primary costing studies conducted in the UK. Parental costs will be obtained from the telephone

interview and will include time off work due to their child's wheeze and time taken to seek medical advice as well as travel expenses and out-of-pocket expenses on additional drugs. The costs associated with non-prescription drugs and visits for private health care will also be recorded. Parental time lost from work and time taken to seek medical advice due to exacerbations will be valued using age- and sex-adjusted average daily wage rates from the Office for National Statistics New Earnings Survey, 2003

10.8.3 Qualitative Analysis

We will use a constant comparison approach to ensure that new themes important to parents can be incorporated as the qualitative study progresses. Translators will be available during interviews as necessary. Transcripts will be imported into qualitative research software (MAXQDA) and analysed by a multidisciplinary team using the framework method, an established methodological approach.

10.9 Interim Analysis

Interim safety analyses will be conducted at 6-monthly intervals (as described above). Efficacy analyses will be conducted on termination of the trial.

10.10 Randomisation and Stratification

Randomisation will be stratified according to section 5.5 of this protocol.

11 Study Finances

11.1 Funding Source

This trial is funded by the National Institute of Health Research/ Medical Research Council Efficacy and Mechanism Evaluation Programme REF; 08/43/03

11.2 Subject expenses and payments

Parents will be offered reimbursement of reasonable travel expenses incurred as a result of their participation in the study.

12 Sponsorship and Indemnity

This trial will be sponsored by Queen Mary University London. The Joint Research Office for Queen Mary University/Barts and The London NHS Trust will arrange for suitable indemnity for negligent harm arising as a result of participation in this study to be in place.

The protocol has been evaluated by the Governance Officer, Queen Mary and assigned an approval number 006983 QM.

13 Publication policy

Any manuscript reporting trial findings will be prepared according to CONSORT guidelines and submitted to peer-reviewed biomedical journals according to ICMJE Uniform Requirements. Authorship will be based on individuals' contribution to study design, conduct, analysis, drafting/revision of manuscript and final approval of the version to be published. Authorship will not necessarily be restricted to individuals named on this protocol; neither is authorship guaranteed to any individual named on this protocol. Contributors who do not meet authorship criteria will be listed in 'Acknowledgements'.

14 References

1. Kuehni CE, Davis A, Brooke AM, Silverman M. Are all wheezing disorders in very young (preschool) children increasing in prevalence? *Lancet*. 2001 Jun 9;357(9271):1821-5.
2. Grigg J, Silverman M. Wheezing disorders in young children: one disease or several phenotypes? *Eur Respir Mon*. 2006;37:6.
3. Davies G, Paton JY, Beaton SJ, Young D, Lenney W. Children admitted with acute wheeze/ asthma during November 1998-2005: A national UK audit. *Archives of disease in childhood*. 2008 May 22;doi. 10.1136/adc.2007.133868:1 - 18.
4. Stevens CA, Wesseldine LJ, Couriel JM, Dyer AJ, Osman LM, Silverman M. Parental education and guided self-management of asthma and wheezing in the pre-school child: a randomised controlled trial. *Thorax*. 2002 Jan;57(1):39-44.
5. Oommen A, Grigg J. Urinary leukotriene E4 in preschool children with acute clinical viral wheeze. *Eur Respir J*. 2003 Jan;21(1):149-54.
6. Knorr B, Franchi LM, Bisgaard H, Vermeulen JH, LeSouef P, Santanello N, et al. Montelukast, a leukotriene receptor antagonist, for the treatment of persistent asthma in children aged 2 to 5 years. *Pediatrics*. 2001 Sep;108(3):E48.
7. Bisgaard H, Zielen S, Garcia-Garcia ML, Johnston SL, Gilles L, Menten J, et al. Montelukast reduces asthma exacerbations in 2- to 5-year-old children with intermittent asthma. *American Journal of Respiratory and Critical Care Medicine*. 2005 Feb 15;171(4):315-22.
8. Robertson CF, Price D, Henry R, Mellis C, Glasgow N, Fitzgerald D, et al. Short-course montelukast for intermittent asthma in children: a randomized controlled trial. *American journal of respiratory and critical care medicine*. 2007 Feb 15;175(4):323-9.
9. Lima JJ. Treatment heterogeneity in asthma: genetics of response to leukotriene modifiers. *Molecular Diagnosis & Therapy*. 2007;11(2):97-104.
10. In KH, Asano K, Beier D, Grobholz J, Finn PW, Silverman EK, et al. Naturally occurring mutations in the human 5-lipoxygenase gene promoter that modify transcription factor binding and reporter gene transcription. *The Journal of Clinical Investigation*. 1997 Mar 1;99(5):1130-7.
11. Lima JJ, Zhang S, Grant A, Shao L, Tantisira KG, Allayee H, et al. Influence of leukotriene pathway polymorphisms on response to montelukast in asthma. *American journal of respiratory and critical care medicine*. 2006 Feb 15;173(4):379-85.
12. MSD Ltd. Summary of Product Characteristics last updated on the eMC: 07/09/2009 <http://emcmedicinesorguk/printfriendlydocument.aspx?documentid=14071&companyid=86>. 2009.