

Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate 5-lipoxygenase (*ALOX5*) promoter genotype: a multicentre, randomised, placebo-controlled trial

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**National Institute for
Health Research**

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Abstract

Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate 5-lipoxygenase (*ALOX5*) promoter genotype: a multicentre, randomised, placebo-controlled trial

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Background: The clinical effectiveness of intermittent montelukast for wheeze in young children is unclear. Previous work has been equivocal. Variation in copy numbers of the specificity protein 1-binding motif in the arachidonate 5-lipoxygenase (*ALOX5*) gene promoter; where the wild type has five copies per allele, denoted here as 5/5, but variant genotypes may have 1–8 copies on each allele, denoted as x/x or $5/x$, influences montelukast efficacy in asthmatic adults. This polymorphism may identify a responsive subgroup within this population.

Objectives: To assess the clinical effectiveness of montelukast in preschool wheezing children. To explore the effect of the *ALOX5* promoter genotype on this effect.

Design: A multicentre, parallel-group, double-blind, randomised, placebo-controlled trial.

Setting: Twenty-one primary care sites and 41 secondary care sites in England and Scotland.

Participants: Children aged 10 months to 5 years with two or more wheeze episodes, one within the last 3 months, stratified by *ALOX5* promoter genotype, either 5/5 or $[5/x + x/x]$. Children with other respiratory vulnerabilities were excluded.

Intervention: Parent-initiated 4 mg oral granules of montelukast or identical placebo administered once daily for 10 days from the onset of every cold or wheeze episode over 12 months.

Main outcome measure: Need for unscheduled medical attendance for wheezing.

Randomisation: Children were stratified by *ALOX5* promoter genotype, either 5/5 or $[5/x + x/x]$, where $x \neq 5$. Children in each stratum were independently randomised to receive montelukast or placebo in a 1 : 1 ratio via a permuted block schedule (size 10). Clinical investigators and parents were blinded to treatment group and genotype stratum.

Methods: Genotype was identified by analysis of salivary deoxyribonucleic acid. Analysis was by intention to treat. Primary outcome data came from treatment diaries, scheduled telephone calls and caregiver records.

Results: A total of 1358 children were randomised to receive montelukast ($n = 669$) or placebo ($n = 677$). Consent was withdrawn for 12 (1%) children. Primary outcome data were available for 1308 (96%) children. There was no difference in unscheduled medical attendances for wheezing episodes between children in the montelukast and placebo groups {mean 2.0 [standard deviation (SD) 2.6] vs. mean 2.3 (SD 2.7) unscheduled medical attendances; incidence rate ratio (IRR) 0.88, 95% confidence interval (CI) 0.77 to 1.01; $p = 0.06$ }. Compared with placebo, unscheduled medical attendances for wheezing episodes were reduced in children given montelukast in the 5/5 stratum [mean 2.0 (SD 2.7) vs. mean 2.4 (SD 3.0) unscheduled medical attendances; IRR 0.80, 95% CI 0.68 to 0.95; $p = 0.01$], but not in those in the [5/x + x/x] stratum [mean 2.0 (SD 2.5) vs. mean 2.0 (SD 2.3) unscheduled medical attendances; IRR 1.03, 95% CI 0.83 to 1.29; $p = 0.79$, p -interaction = 0.08]. We recorded one serious adverse event: a skin reaction in a child allocated to placebo.

Interpretation: There is no clear benefit of intermittent montelukast in young children with wheeze. However, the 5/5 *ALOX5* promoter genotype might identify a montelukast-responsive subgroup.

Limitations: The study lacks power to confirm the validity of the suggested genotype stratum effect. Additionally, the effect is contrary to that hypothesised and is not supported by urinary data. We could not robustly measure treatment compliance.

Future work: Future work should test the stratum effect with a repeat trial in the apparently more responsive (5/5) stratum only.

Study registration: ClinicalTrials.gov NCT01142505.

Funding: This project was funded by the Efficacy and Mechanism Evaluation programme, a Medical Research Council and National Institute for Health Research partnership.

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Glossary

Arachidonate 5-lipoxygenase Membrane-bound 5-lipoxygenase.

Specificity protein 1 A zinc finger deoxyribonucleic acid transcription factor.

Tag single nucleotide polymorphism A single nucleotide polymorphism in a region of the genome with high-linkage disequilibrium that serves as a representative marker for a group of single nucleotide polymorphisms called a haplotype.

List of abbreviations

AE	adverse event	LTA ₄	leukotriene A ₄
ALOX5	arachidonate 5-lipoxygenase	LTE ₄	leukotriene E ₄
ALOX5AP	arachidonate 5-lipoxygenase-activating protein	MHRA	Medicines and Healthcare products Regulatory Agency
CI	confidence interval	PI	principal investigator
cLT	cysteinyl leukotriene	PIS	patient information sheet
CRF	case report form	SAE	serious adverse event
DNA	deoxyribonucleic acid	SD	standard deviation
DSMC	Data and Safety Monitoring Committee	SNP	single nucleotide polymorphism
GP	general practitioner	SP1	specificity protein 1
IMP	investigational medicinal product	T0	time of first IMP dispensing
IRR	incidence rate ratio	USMA	unscheduled medical attendance
ITT	intention to treat	WAIT	Wheeze And Intermittent Treatment trial
LT	leukotriene		

Plain English summary

Background

Young children who wheeze can become very ill and may require large amounts of care from their families, doctors and nurses. No treatment has been shown to work very well for these children. Montelukast is an established medicine that is safe and easy to give to children.

What did we want to find out?

We wanted to see if montelukast might help in this group of children. We wanted to see whether or not children with slightly different genes might do better with montelukast than others.

What did we do?

We ran a research study comparing montelukast with a dummy medicine (referred to as placebo). We gave parents a box of medicine but did not tell them whether it was montelukast or placebo. We asked them to start giving it to their child as soon as a cold or wheezing episode began and to continue it for 10 days. We measured the number of times children needed to see a doctor or nurse in an unplanned way over the following year.

What did we find?

Montelukast did not seem to help young wheezing children any more than placebo did. It might have worked better in children with slightly different genes but we could not be certain from this study.

What does this mean?

Montelukast does not seem to be helpful for all young children who wheeze. We need to do another study to see if it really does work better in the group of children with slightly different genes.

Scientific summary

Background

Wheeze in preschool children is a common and important cause of morbidity, with an associated social and economic burden through strain on health services and parental resources. Current evidence does not support the use of oral corticosteroids in this population because of a lack of efficacy in reducing hospital stay and demonstrable oral treatment-associated morbidity when used to excess. The majority of children wheeze only with colds, with few or no symptoms in the interim. There is a need for a treatment that can be administered effectively during symptomatic episodes but can be discontinued when children are well.

The cysteinyl leukotrienes (cLTs) are inflammatory mediators derived from arachidonic acid that have potent bronchodilator effects. Previous work has shown a transient increase in leukotriene (LT) production [measured as urinary leukotriene E₄ (LTE₄)] in preschool children during acute wheezing episodes, implicating them as the transient mediator for episodic viral wheeze in this population.

Montelukast (Singulair[®], Merck Sharp & Dohme Ltd) is the only LT receptor antagonist licensed for use in children. It is a competitive inhibitor of the cLT receptor binding site and prevents the downstream bronchoconstrictor and pro-inflammatory effects of the cLTs. Moreover, it is safe and orally available, with an appropriate half-life and posology suitable for all ages. Previous work has suggested a role for intermittent therapy in the management of acute childhood wheeze, but the effects have been modest. Analysis of adult trials suggests that variation in copy number of a guanine–cytosine-rich specific protein 1-binding motif (the wild type has five copies) in the promoter region of the arachidonate 5-lipoxygenase (*ALOX5*) gene may influence response to montelukast, presumably by altering baseline or exacerbation-related LT production.

Primary objectives

1. To assess the efficacy of parent-initiated intermittent montelukast for the reduction of unscheduled medical attendances for preschool wheeze.
2. To explore the role of the *ALOX5* promoter genotype in montelukast efficacy.

Secondary objectives

1. To assess the impact of intermittent montelukast on respiratory morbidity.
2. To assess the impact of montelukast on health service usage.
3. To assess the impact of intermittent montelukast on adverse events (AEs).
4. To assess the impact of intermittent montelukast on concomitant medication use.
5. To gather exploratory data on related LT pathway genes.
6. To gather exploratory data on urinary LT/eicosanoid output.
7. To assess impact of intermittent montelukast on economic outcomes.
8. To assess qualitative outcomes related to parent-initiated intermittent therapy for preschool wheeze and participation in a genetically stratified interventional trial.

Methods

Overall, it was hypothesised that montelukast would be moderately effective but that a subgroup of children with a variant (non-5 repeat) allele on one or both chromosomes would have a greater response to montelukast, manifesting as a decreased need for unscheduled medical attention compared with their peers when treated with montelukast.

To test this hypothesis we recruited children from primary and secondary care settings. Eligible children were aged 10 months to 5 years, had had two or more previous episodes of wheeze, with one occurring within the previous 3 months, and had no associated significant respiratory morbidity. Younger and older children were excluded so as not to confuse the pathology studied with viral bronchiolitis or classical asthma. At enrolment, children provided salivary deoxyribonucleic acid and were stratified by *ALOX5* genotype, with one stratum comprising those with five copies of the *ALOX5* promoter on each allele and the other comprising all those with one or more non-5 repeat allele. The two strata were subsequently independently randomised in a 1 : 1 ratio (randomly permuted blocks of 10) to receive montelukast oral granules or identical placebo every day for 10 days from the start of a cold or wheezing episode. Need for unscheduled medical attention over a period of 12 months was assessed as the primary outcome. Outcome data were collected via a treatment diary completed with every course of investigational medicinal product and via a bimonthly investigator telephone call which additionally screened for AEs.

We also measured urinary LTE_4 at baseline and during exacerbation (where possible) to provide pathophysiological corroboration of any associations observed. Urine was collected fresh into a universal container and placed on ice before being transferred within 48 hours to a freezer at -70°C . Urine samples were then batch analysed using high-performance liquid chromatography–tandem mass spectrometry for a number of eicosanoid mediators, with results indexed to urinary creatinine.

A subset of recruits underwent semistructured qualitative interviews conducted by an experienced qualitative researcher, with an interpreter where required. Questions addressed background information about the child and family, as well as parental experiences and attitudes to their role in the trial. Interviews were audio-recorded, transcribed and imported into Nvivo9™ (QSR International, Pty Ltd, VIC, Australia; a qualitative data analysis program) for analysis.

Results

Out of the 1358 subjects recruited, data on which to assess the primary outcome were available for 1308 (96%) subjects. Analysis was by intention to treat. Overall, montelukast did not outperform placebo in intermittent usage for preschool wheeze [incidence rate ratio (IRR) = 0.88; $p = 0.06$]. In children treated with montelukast, use of rescue oral corticosteroids, a recognised marker of severity, was marginally reduced (IRR 0.75; $p = 0.03$); however, the study was not adequately powered to robustly detect such a change.

Analysis by genotype suggested an improved montelukast effect (contrary to our hypothesis, but in keeping with certain earlier work) in the wild-type (5/5) stratum (IRR = 0.80; $p = 0.01$). When subject to more detailed scrutiny this observation was not statistically robust, with a p -value for interaction of only 0.08. No effect was seen when the primary outcome was analysed by use of inhaled corticosteroids, wheezing phenotype or alternative genotype grouping x/x versus $[x/5 + 5/5]$.

Urinary eicosanoid results

Leukotriene E_4 appeared higher in subjects with two variant alleles (x/x) ($p < 0.05$). This was inconsistent with the direction of association predicted by the possible improved montelukast effect in the 5/5 population. Analysis of urinary cotinine and urinary LTE_4 by LT pathway single nucleotide polymorphisms (SNPs), etc., remains ongoing.

Qualitative results

Bangladeshi families were relatively reluctant to participate in the qualitative study, despite strong engagement with the parent study. Anxiety related to wheezing was a common primary motive for trial enrolment. Parents viewed the trial as a route to improved treatment. Verbal delivery of trial information appeared to be more effective than study literature, especially for Bangladeshi families, with low parental literacy and high levels of trust in medical professionals potential contributors to this effect. All ethnic groups expressed a low understanding and/or retention of essential study concepts such as randomisation and genetic testing.

Conclusions

This study does not support the routine use of intermittent montelukast in preschool wheezing children. It does not speak to the value of continuous montelukast in this population; neither does it preclude the consideration of short-term therapeutic trials on an individual patient basis in this context. The suggested superior montelukast response in the 5/5 stratum is of interest but is not robust insofar as the test of interaction does not meet statistical significance and the finding contradicts both the a priori hypothesis and the urinary LTE₄ data.

Future research

The effect seen in the 5/5 stratum will be prospectively evaluated in a study population comprising children with only wild-type (5/5) alleles. Should this study be negative it remains possible that a montelukast-responsive subgroup exists. In the course of this trial we have collected data on more than 100 LT pathway SNPs with associated urinary eicosanoid profiles and demographic data. Subsequent work will interrogate this data set for candidate genes and biomarkers that might identify any responsive subgroup with a view to hypothesis generation for future large-scale trials of stratified therapy in preschool wheeze.

Trial registration

This study is registered as ClinicalTrials.gov NCT01142505.

Funding

Funding for this study was provided by the Efficacy and Mechanism Evaluation programme of the National Institute for Health Research.

Chapter 1 Introduction

Preschool wheeze

One-quarter of preschool children between 1 and 5 years 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 two clinical patterns of preschool wheeze: (1) episodic virus-triggered wheeze, which affects the majority of wheezing children; and (2) multitrigger wheeze, which affects the minority of children.

Montelukast in preschool wheeze

A promising therapy for both clinical phenotypes of wheeze is montelukast (Singulair[®], Merck Sharp & Dohme Ltd), currently the only cysteinyl leukotriene (cLT) receptor antagonist licensed for use in young children. This beneficial effect of inhibition of cLTs, a class of potent bronchoconstrictors, in preschool wheeze was suggested by a study of urinary cLTs, where levels of urinary leukotriene E₄ (LTE₄) were elevated during acute attacks of preschool wheeze and then, on convalescence, fell into the normal range.⁵ A study relevant to 'multitrigger' preschool wheeze is a randomised controlled trial of 689 young children in whom regular oral montelukast given over a 12-month period reduced the rate of exacerbations by 30%.⁶ In the case of 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. We recruited a heterogeneous group of children aged between 2 and 14 years with intermittent asthma into a 12-month placebo-controlled randomised controlled trial of oral montelukast (the Pre-Empty study).⁸ 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 The montelukast-treated group had 162 unscheduled health-care resource utilisations for wheeze, compared with 288 in the placebo group, and symptoms were significantly reduced by 14% in the montelukast-treated group.⁸ As intermittent therapy may be effective in preschool wheeze, the aim of the Wheeze And Intermittent Treatment (WAIT) trial was to assess whether or not parent-initiated montelukast therapy is efficacious in this condition.

Genetics of montelukast response and study rationale

The beneficial effect of montelukast, albeit consistent, is clinically relatively modest.⁸ The overall modest benefit of montelukast is thought to be due to marked heterogeneity of response: that is, some children respond very well while others do not respond at all. One explanation for this marked heterogeneity in response is variation in the genes coding for components of the leukotriene (LT) pathway.⁹ The first step in LT production is the creation of leukotriene A₄ (LTA₄) by arachidonate 5-lipoxygenase (ALOX5; other names for ALOX5 are 5-lipoxygenase and LTA₄ synthase) and ALOX5-activating protein (encoded by the *ALOX5AP* gene). The regulatory domain of ALOX5 controls leukotriene synthesis by catalysing the conversion of arachidonic acid to 5(S)-hydroxyeicosatetraenoic acid and further dehydration to LTA₄. The *ALOX5* promoter polymorphism results in a variation in the number of specificity protein 1 (SP1) transcription factor-binding motifs – which alters transcription factor binding and influences *ALOX5* gene expression.¹⁰ The five SP1 repeats in the *ALOX5* gene 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 $x \neq 5$) have a 73% reduction in the risk of having an asthma attack

on montelukast compared with homozygotes for the five-repeat (5/5; wild-type) allele. Therefore, we hypothesised that, overall, parent-initiated montelukast therapy in preschool wheeze would be clinically moderately effective, but that there would be a highly responsive subgroup of children defined by *ALOX5* polymorphism status (i.e. carrying a variant number of repeats on at least one allele). In this trial we therefore included 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.

Study objectives

Primary objectives

1. To assess the efficacy of parent-initiated intermittent montelukast for the reduction of unscheduled medical attendances for preschool wheeze.
2. To explore the role of the *ALOX5* promoter genotype in montelukast efficacy.

Secondary objectives

1. To assess the impact on intermittent montelukast on respiratory morbidity.
2. To assess the impact of montelukast on health service usage.
3. To assess the impact of intermittent montelukast on concomitant medication use.
4. To assess the impact of intermittent montelukast on adverse events (AEs).
5. To gather exploratory data on related LT pathway genes.
6. To gather exploratory data on urinary LT/eicosanoid output.
7. To assess the impact of intermittent montelukast on economic outcomes.
8. To assess qualitative outcomes related to parent-initiated intermittent therapy for preschool wheeze and participation in a genetically stratified interventional trial.

Chapter 2 Methods

Overall study design

This was a double-blind, randomised, placebo-controlled trial of intermittent montelukast therapy. The study population comprised preschool children (aged 10 months to 5 years, inclusive) who had experienced two previous episodes of wheeze. Target accrual was 1300 patients. Eligibility criteria were as stated in *Participants*. An overview is provided in *Figure 1*. Patients were recruited in primary and secondary care, and were stratified according to *ALOX5* promoter genotype. Patients were then randomised within their stratum to receive either intermittent montelukast or placebo for 10 days from the start of a viral cold or wheezing episode, with the need for unscheduled medical attention monitored over a 12-month follow-up period.

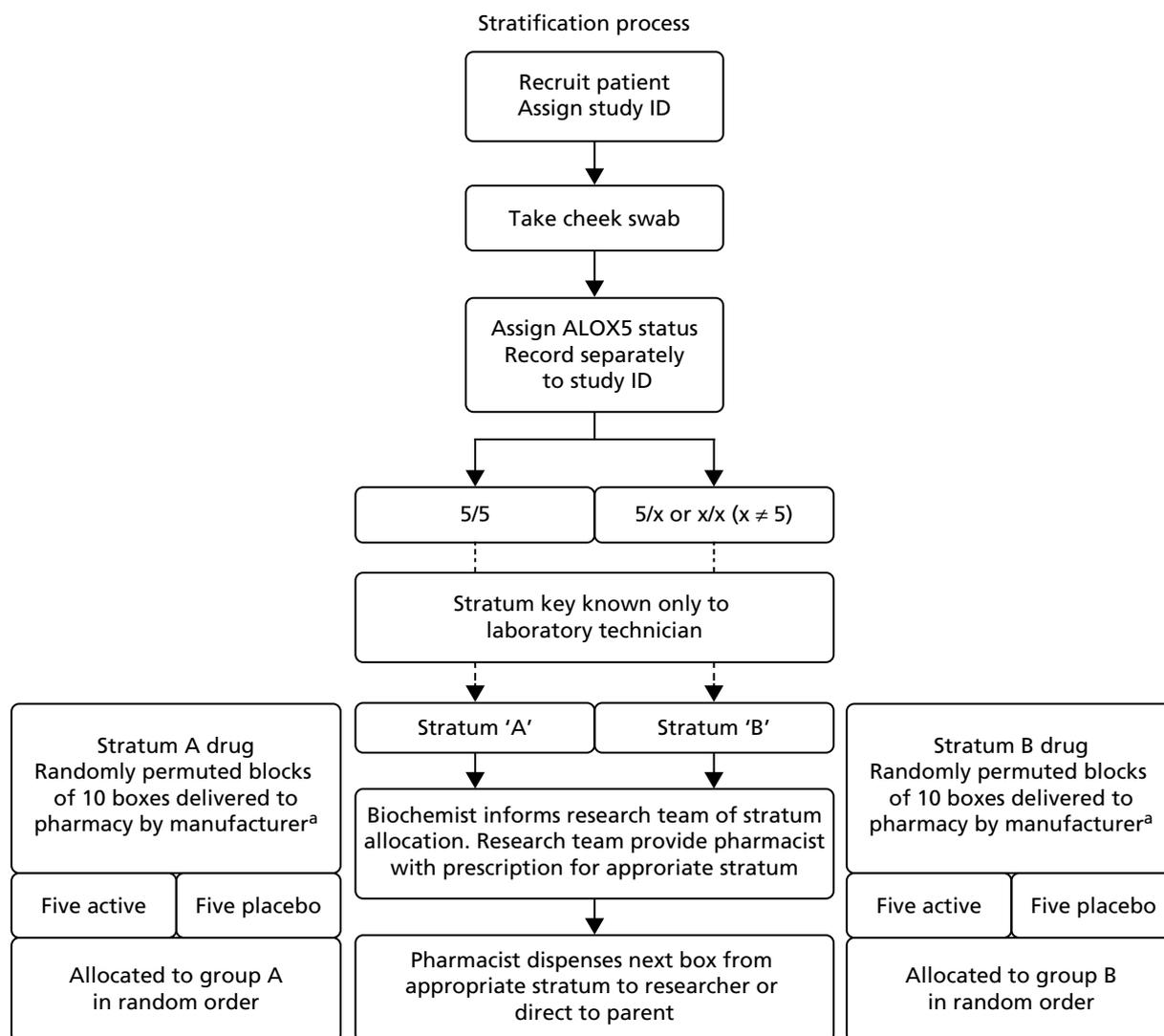


FIGURE 1 Schematic chart of trial protocol. a, Subset of participants. ID, identification number.

Participants

Eligibility criteria

Patients were eligible for the study if they fulfilled the following criteria:

- aged ≥ 10 months and ≤ 5 years on the day of the first dose of the investigational medicinal product (IMP)
- two or more attacks of parent-reported wheeze
- at least one attack with wheeze validated by a clinician (nursing or medical)
- the most recent attack within the last 3 months
- contactable by telephone and able to attend one face-to-face review
- parent or guardian able to give written informed consent for their child to participate in the study.

Exclusion criteria

The following characteristics rendered patients ineligible for the study:

- 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 or sickle cell disease
- 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.

Selection of study population

As indicated previously, wheezing is common in otherwise healthy preschool children; however, safe effective treatment options are limited. We therefore sought to conduct a pragmatic trial with the widest possible useful application. Thus, participants were not limited in terms of wheeze severity or concomitant medications, notwithstanding the prohibition of regular montelukast. We did not include children aged less than 10 months and greater than 5 years in order to exclude children with classical bronchiolitic or asthmatic phenotypes, for which treatment strategies differ.

Recruitment and patient journey

Recruitment setting

Participants were identified in primary and secondary care centres. Recruitment was planned to encompass only three secondary care centres (the Royal London Hospital, University Hospital Leicester and the Royal Aberdeen Children's Hospital), but increased to 41 secondary care centres in England and Scotland (see *Acknowledgements*) in response to observed recruitment rates.

Invitation of potential study participants to attend screening visit

A member of the child's usual general practitioner (GP) care team or the hospital paediatric team (as appropriate) identified potentially eligible children based on age and history of wheeze from reviewing surgery and emergency department records. The parent/guardian was then approached in person or via a posted invitation letter and/or information sheet, to ask if they would like to be contacted about the study by a member of the research team. Individuals who agreed to be contacted about the study were then contacted by a research nurse or research assistant, who briefly described the study to them, and asked them if they would like to read a patient information sheet (PIS; see *Appendix 1*) if not already given. The research nurse or research assistant then provided a PIS to parents who expressed an interest in the study; those who subsequently confirmed their interest in participation were offered a screening appointment at a study site. A second invitation letter was posted to individuals who did not respond to the first invitation letter.

Screening visit 2 weeks prior to first investigational medicinal product dispensing

At the screening visit, an investigator, or a suitably trained person delegated by the investigator (a research nurse or a research assistant who had attended a UK good clinical practice training course), gave an adequate explanation of the aims, methods, anticipated benefits and potential hazards of the study. The eligibility of children to participate in the study was assessed in accordance with the criteria documented in *Participants*. The investigator then obtained written informed consent (see *Appendix 2*) from the parent or guardian prior to participation in this study. A period of at least 24 hours, or an overnight stay in hospital (for patients recruited during an acute admission), was required for consideration by the parent or guardian before they gave consent to enter the study. During the consent process it was made clear that parents or guardians were completely free to refuse to enter the study or to withdraw at any time during the study, for any reason. The parents of all eligible children were asked to complete baseline assessments of their child's wheeze status including recording of baseline demographic and clinical data and details of concomitant medications (see *Appendix 3*). They also underwent measurement of weight and height, provided a salivary sample for genotyping (see *Salivary sample*) and gave a urine sample for LT analysis (see *Urine sample* and *Appendix 4*). A follow-up appointment was arranged for the issue of the IMP.

Stratification (–1 week)

Saliva samples were posted to the Blizzard Institute, Queen Mary University of London, London, UK, where deoxyribonucleic acid (DNA) was extracted and children assigned either to the *ALOX5* promoter polymorphism 5/5 stratum or to the [5/x + x/x] stratum, depending on the number of copies of the *ALOX5* promoter polymorphism they had on each allele. Extracted DNA was stored in a freezer at –70 °C for later batch analysis of > 150 polymorphisms in 10 genes encoding components of the LT biosynthetic pathway and the LT receptors. The study pharmacist then randomised subjects within their strata, and the corresponding box of active or placebo medication was dispensed for issue at the time of first IMP dispensing (T0) visit (see *Figures 1* and *2*).

Method of assigning patients to treatment groups: randomisation

Nova Laboratories Ltd (Novalabs, Leicester, UK) prepared the IMP for this trial. Preparation was intended to comprise 6-monthly batches tailored to recruitment rate, with an expectation that 1300 boxes of 50 sachets containing active montelukast and 1300 containing placebo would be produced at a minimum. However, a national shortage of montelukast necessitated a production of boxes containing between 20 and 50 sachets, so as to maintain supply and not compromise recruitment and subject retention. The change in box size received approval from the Medicines and Healthcare products Regulatory Agency (MHRA) prior to implementation. Boxes were allocated randomisation numbers in blocks of 10 using a computer-generated random sequence. Novalabs was responsible for generation of the random number sequence and labelling of boxes. Boxes bearing randomisation numbers were initially delivered to the pharmacy at participating sites. Subsequently, the expansion of site numbers prompted a move to central randomisation and distribution of IMP (from the sponsor pharmacy to participating sites). Novalabs produced additional boxes of IMP for those children whose IMP supply was lost, reached expiry or was exhausted such that they required additional boxes during the 1-year follow-up period. Clinicians remained blinded to allocation throughout.

Randomisation was stratified according to the *ALOX5* promoter polymorphism status yielding two genotype groups:

- Group 1: children with the 5/5 *ALOX5* promoter polymorphism genotype.
- Group 2: children with the [5/x + x/x] *ALOX5* promoter polymorphism genotype, where $x \neq 5$ SP1 repeats. (Groups were referred to as stratum A or B.)

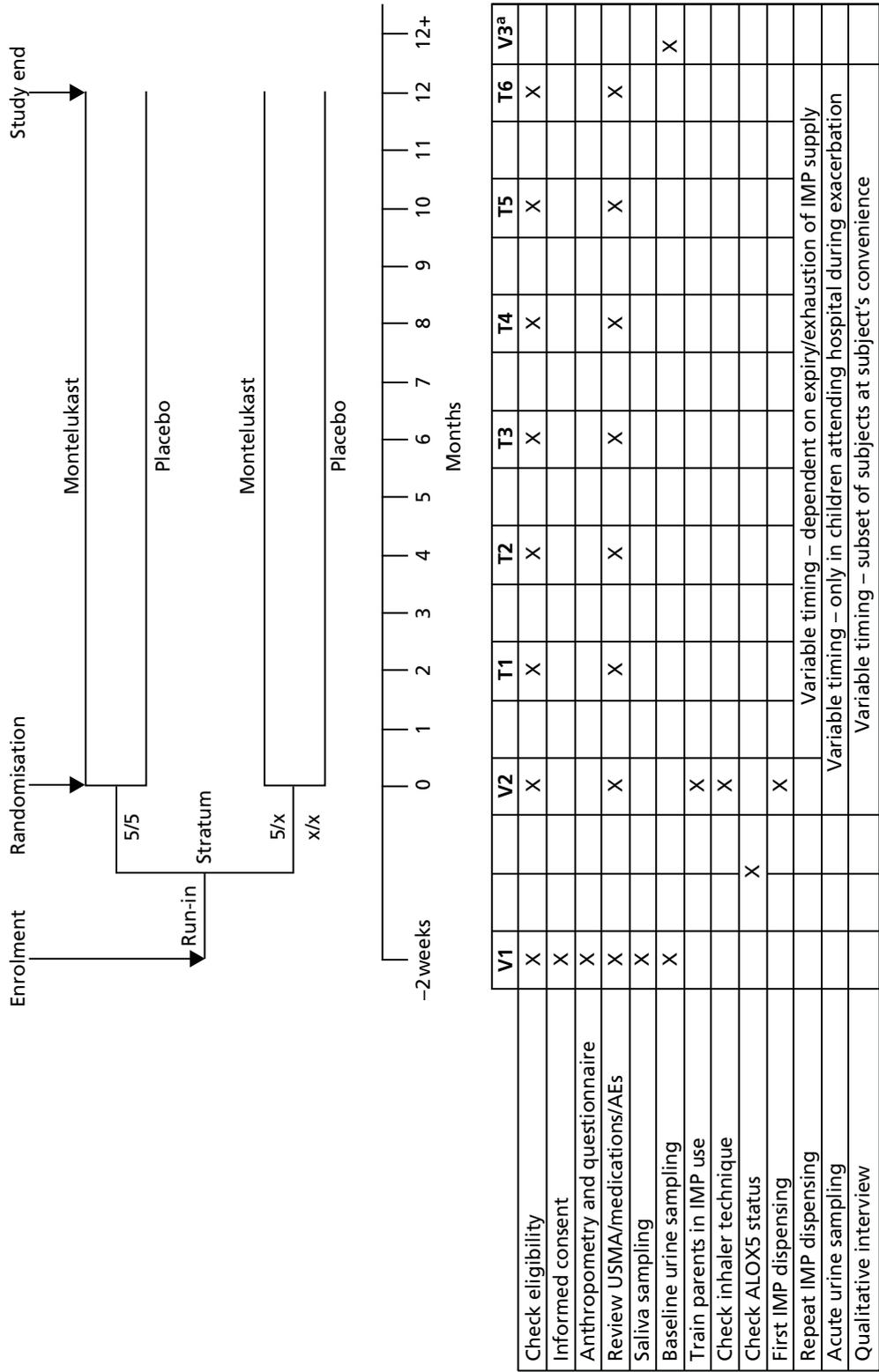


FIGURE 2 Stratification and randomisation schematic. a, Randomisation key provided by manufacturer, held in sealed envelope by pharmacist and Data Monitoring Committee. T, telephone call; USMA, unscheduled medical attendance; V, visit.

Children in each of these two genotype groups (strata) were assigned consecutive randomisation numbers from randomised permuted blocks of 10 representing the randomisation numbers on the IMP boxes. Within each block, equal numbers of children were randomly allocated to placebo and active treatment. When all numbers from the first block had been assigned, a new block of randomisation numbers was allocated to that stratum until a total of 1300 children in the two strata combined had been assigned a randomisation number (see *Figure 2*).

Blinding

Novalabs produced a corresponding randomisation code denoting whether a given IMP box contained active medication or placebo. This was kept sealed and held only by the clinical trials pharmacist and a member of the Independent Data and Safety Monitoring Committee (DSMC); in this way all other clinical investigators and participants remained blinded to treatment allocation.

T0 visit (0 months)

The research nurse or research assistant met with parents, confirmed eligibility and issued parents a box containing IMP sachets. Parents were taught how to use the IMP. They were also provided with one study diary card (see *Appendix 3*) and one Freepost return envelope (addressed to the sponsor organisation) per 10 sachets. Parents were asked to return completed diary cards and empty sachets at completion of a course of IMP. Each diary card recorded clinical and IMP usage data for the 10 days of the IMP course.

Telephone calls at 2, 4, 6, 8, 10 and 12 months

At approximately 2-month intervals following the T0 visit, a research nurse or research assistant telephoned the subject's carer to check if the parent had initiated the IMP, the numbers of days the IMP had been used, the use of health-care resources, any concomitant medications, any procedures, number of days lost from childcare and parent days lost from work. Any AEs experienced were also recorded.

Qualitative interview visit (variable timing)

Qualitative interviews were conducted in a subgroup of families recruited at the sponsor site. The aim of these was to establish attitudes towards genetic testing to guide personalised therapy, acceptability of parent-initiated therapy for preschool wheeze, the expected advantages and disadvantages of using the IMP, and their views on the consent process and PIS. Interviews included either or both parents and, where possible, were conducted at the parental home. Interviewing, transcribing and analysis of interviews was performed by a researcher skilled in qualitative research, in the presence of a translator where necessary.

Withdrawal of patients from therapy or assessment

Patients were free to withdraw from the study at any time without giving a reason. Patients were advised that if they requested to withdraw from the study, at any time during the trial, then this would have no negative consequences. Investigators could also withdraw patients from the trial if they deemed it appropriate for safety or ethical reasons or if it was considered to be detrimental to the well-being of the patient. Where possible, patients who withdrew or were withdrawn underwent a final telephone or face-to-face evaluation. Those participants who withdrew and provided permission to use their data were included in the analysis up to the point of withdrawal. Full documentation was made of any withdrawals that occurred during the study in the case report form (CRF). The investigator documented the date and time of the withdrawal and results of any assessments made at this time. If the patient withdrew because of an AE or a serious adverse event (SAE), then details were forwarded to the Research Ethics Committee, as required, and to the sponsor, who forwarded details to the regulatory authorities as appropriate.

Interventions

Details of the IMP are as shown in *Table 1*.

Administration of the investigational medicinal product

Subsequent to stratification, children were randomised within their stratum to receive either montelukast or identical placebo. All study treatment was dispensed from the study pharmacy either directly to the patient carer or to the study investigator or designated member of staff for distribution to the carer. The IMP was administered unsupervised by the patients' carers in their usual place of residence. The IMP was presented as white granules administered either directly into the child's mouth, or mixed with a spoonful of cold or room-temperature soft food (e.g. apple sauce, ice cream, carrots and rice). The IMP was used according to the primary manufacturer's instructions. Specifically, parents were advised not to open the sachet containing the granules until ready to use. After opening the sachet, the full dose of granules was administered within 15 minutes. If mixed with food, the granules would not be stored for future use. The granules were not intended to be dissolved in liquid for administration; however, liquids could be taken subsequent to administration. The granules could be administered without regard to the timing of food ingestion. The dose was one 4 mg sachet per day, started when the child had evidence of a viral cold or had a wheeze, and stopped after 10 days. Children were permitted to commence a second course of IMP should the wheeze not resolve within 10 days. If a child vomited after the administration of the IMP, no additional dose was given and parents recorded this on the diary chart.

Selection of doses in the study

Montelukast is an established medication in this patient population with an accepted dosing of 4 mg daily. The granule formulation was selected to achieve the broadest tolerability across the preschool age group. The IMP was administered at the first sign of a cold and continued for 10 days to give the best chance of covering the entire duration of any virus-induced LTE₄ overproduction. There was no variation of dosing strategy or posology between patients.

TABLE 1 Particulars of the IMP

Detail	Active drug	Placebo
Trade name	Singulair granules	Mannitol EP (PEARLITOL® 200 SD)
Composition	4 mg of montelukast sodium (which is equivalent to 4 mg of montelukast) granules with mannitol excipient	Mannitol granules
ATC code	R03DC03	Not applicable; drug master file lodged with the EP commission
Pharmaceutical form	Granules	Granules
Dosage regimen	One sachet to be given once a day at the start of a cold or wheezy episode, and continued for 10 days	One sachet to be given once a day at the start of a cold or wheezy episode, and continued for 10 days
Route of administration	Oral	Oral
Manufacturer	Merck Sharp & Dohme Ltd (purchased on the open market)	Roquette Pharma

ATC, Anatomical Therapeutic Chemical; EP, European Pharmacopoeia; SD, spray dried.

Prior and concomitant therapy

Subjects were eligible for the study as long as they were not taking regular montelukast. No limitations were placed on concomitant medications; however, medications were recorded on the CRFs at study entry and during follow-up.

Other measurements

Safety measurements

Montelukast is an established drug with a good safety profile. Safety assessments were limited to standard AE reporting, with patterns monitored by the DSMC.

Other measurements

Subjects underwent the following assessments during the study (see *Figure 1* for timings).

Weight

Weight in light clothing was measured with weighing scales and recorded in kilograms.

Height

Height without shoes was measured using a stadiometer.

Salivary sample

A sample of DNA was collected from saliva using the Oragene™ infant sponge system (DNA Genotek Inc., Kanata, ON, Canada). The sponge tips were cut into an Oragene DNA kit (DNA Genotek Inc., Kanata, ON, Canada) to preserve the DNA and prevent bacterial growth. This method yields high-quality DNA and eliminates the need for traditional cheek-scraping methods.

The *ALOX5* polymorphism status was determined within 1 week of sampling in the sponsor's laboratory. DNA was extracted in accordance with a standard operating procedure and the manufacturer's instructions (DNA Genotek Inc., Kanata, ON, Canada). Products of the polymerase chain reaction were analysed by capillary electrophoresis on a 3130xl Genetic Analyser [Applied Biosystems (a brand of Thermo Fisher Scientific), MA, USA]. Polymerase chain reaction amplicons were obtained, varying in size depending on the copy number of the repeat sequence, and were visualised using GeneMapper v4 software [Applied Biosystems (a brand of Thermo Fisher Scientific), MA, USA]. Genotypes were called from duplicate amplifications, with respect to standards run on each plate that are verified by direct DNA sequence analysis. In addition, 150 polymorphisms in 10 genes encoding components of the LT biosynthetic pathway and the LT receptors were assessed: *ALOX5*, *ALOX5AP*, *LTC4S* (leukotriene C₄ synthase), *CYSLTR1* (cysteinyl leukotriene receptor 1), *CYSLTR2* (cysteinyl leukotriene receptor 2), *PLA2G4A* (phospholipase A₂ Group 4A), *LTA4H* (leukotriene H₄ hydrolase), *LTB4R1* (leukotriene B₄ receptor 1), *LTB4R2* (leukotriene B₄ receptor 2) and *MRP* (ribonucleic acid component of mitochondrial ribonucleic acid processing endoribonuclease). These included all single nucleotide polymorphisms (SNPs) located in promoter regions, exons, intron–exon boundaries and the SNPs within the *ALOX5AP* haplotypes (referred to as HapA and HapB). Additional tag SNPs were selected using the LDselect algorithm (version 1; University of Washington, Seattle, WA, USA) 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 the National Institute of Environmental Health Sciences SNPs databases. SNP genotyping was carried out using the KBioscience™ competitive allele-specific polymerase chain reaction (KASPar) method (KBioscience Ltd, Hoddesdon, UK).

Urine sample

A urine sample was to be collected from children in spontaneously voided urine using an age-appropriate method into a sterile receptacle. A first urine sample was obtained when patients were well and a second, where possible, during an acute wheezing illness. In a subset of children whose parents agreed, repeat 'well' urine samples were obtained on study exit to assess repeatability. Urinary leukotriene level was assessed using a high-performance liquid chromatography–tandem mass spectrometry technique (see *Appendix 4*). Values were indexed to urinary creatinine.

Appropriateness of measurements

The primary outcome measure was one that is of importance to patient/carers, clinicians and policy-makers and was deemed more robust to local variations in treatment practices than other measures. It has previously been used in similar studies in this population and is measurable without undue patient inconvenience.

Urine LTE₄ level reflects leukotriene metabolism and has been correlated with asthma severity and bronchoconstriction.¹² A significant correlation with montelukast efficacy would provide both a non-invasive and inexpensive marker to guide treatment.

The anthropomorphic and urine measurements are of minimal inconvenience, while the Oragene saliva kit (DNA Genotek Inc., ON, Canada) yields high concentrations of DNA and is well tolerated by patients.

Data quality assurance

Data from source material and CRFs were entered into a secure electronic database managed by a clinical trials unit data manager. Prior to analysis, 10% of records were randomly checked against source data by the co-ordinating principal investigator (PI), with good concordance. All available data can be obtained from the corresponding author.

Primary outcome

The number of times a child attends for an unscheduled medical opinion with respiratory problems over a 12-month period was recorded on diary cards and in bimonthly telephone calls, and was confirmed from clinical records.

Secondary outcomes

The following outcomes were assessed as indicated via diary card, telephone call and health records.

Respiratory morbidity

- 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 as recorded in the diary card.
- Severity of episodes as recorded in the diary card.
- Parents' overall impression of efficacy of the IMP.

Health service use

- Unscheduled GP consultation with exacerbation of wheeze, expressed as time from randomisation to first attendance and annual attendance rate.
- Accident and emergency 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

- SAEs.
- 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 mean use per wheeze episode as recorded in the diary card by a parent/guardian.

Inflammatory outcomes

Association between baseline urinary cLT level and:

- arachidonate 5-lipoxygenase status
- other polymorphisms of leukotriene genes
- previous history of virus-triggered episodic and multitrigger wheeze
- responsiveness to montelukast
- acute history of wheeze
- urinary cotinine.

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 LT synthesis, metabolism and activity.

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 the PIS.

Study definitions

Need for unscheduled medical attention

This was defined as an episode requiring an unscheduled attendance at either a general practice or an accident and emergency department, or a combination of both, where wheeze is diagnosed by a clinician.

Time from randomisation to first attack of severe wheeze

This was defined as the number of days from the date of administration of first dose of the IMP to the first date on which a wheeze exacerbation meets the criteria for severity stated in *Need for unscheduled medical attention*.

Number of days with parent-reported wheeze

This was defined as the number of days with wheeze over the 12-month trial period obtained by telephone contact with the researcher and recorded in the diary card.

Use of inhaled relief medication

This was expressed as the total number of occasions inhaled relief medication was used over the 12-month trial period. The mean number per wheeze episode was obtained from the number of actuations calculated from records in the diary card.

Statistical methods

Statistical analysis plan

The statistical analysis plan is available in *Appendix 5*. The analysis was based on intention-to-treat (ITT) principles.

Determination of sample size

This trial was powered to detect a clinically significant difference in the number of attacks of wheeze between the intervention and control arms. The trial also had power to detect large differential responsiveness (in terms of the primary outcome) to montelukast in the stratum with the *ALOX5* promoter polymorphism 5/5 compared with the stratum with the *ALOX5* [5/x + x/x] genotype.

Prior to the start of the trial, data on the mean (0.76) and standard deviation (SD) (1.22) number of attacks of wheeze came from the UK GP Research Database on courses of oral steroids (a proxy for number of episodes). These data followed an overdispersed Poisson distribution. To take account of this, a Markov chain Monte Carlo simulation in WinBUGS™ (version 1.4.3; MRC Biostatistics Unit, Cambridge, UK) was used to estimate the sample sizes required to detect a 33% drop in attack rate requiring medical attention, with a power of 90%, a significance level of 5% and a 6% loss to follow-up. In total, 1050 children were required. 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 also gave just over 80% power at the 5% significance level to detect an interaction between treatment and genotype if the effect was a 60% reduction in the [5/x + x/x] genotype and a 20% reduction in the 5/5 stratum. In addition, assuming a 6% dropout, 1300 children needed to be recruited.

Analysis of primary end points

Initial analyses were performed according to ITT for all participants with outcome data. Per-protocol efficacy analyses were also performed, excluding data collected after discontinuation of the IMP for those participants who discontinued using the IMP. We used Poisson regression with a random effect representing individuals to account for overdispersion. Fixed effects represent the stratification factor (*ALOX5* promoter) and treatment centre. The incidence rate ratio (IRR) (relative risk) and 95% confidence interval (CI) were calculated. The analysis was to be conducted in Stata V12 (StataCorp, College Station, TX, USA). To test for a differential effect by stratum an interaction between stratum and treatment was fitted to this model as described in *Genetic analysis*.

Analysis of secondary end points

A Poisson regression analysis with a random effect for individuals to allow for overdispersion was applied to determine the influence of treatment allocation on number of days with parent-reported wheeze, number of hospital attendances and number of admissions to hospital. An IRR for each factor is presented with 95% CIs.

Time to first attack of wheeze was analysed using a log-rank test with adjustment for clustering and (where hazards are proportional) Cox's proportional hazards models adjusting for clustering. In the Cox model, stratum and centre were included as covariates.

Other continuous variables were analysed with analysis of covariance. Dichotomous variables were analysed with logistic regression analysis. AEs are analysed with descriptive statistics.

Genetic analysis

To assess the difference in responsiveness to montelukast in the two *ALOX5* strata, an interaction term was fitted for each treatment arm to test for the interaction between montelukast and stratum in our main model. The associations between genotype and clinical phenotype, urinary LT level and clinical outcome were reported. To test the polymorphisms in each gene in combination, a composite likelihood approach was used, which combines the regression coefficients for all polymorphisms at each locus. Analysis of clinical effectiveness (utility) of stratification of *ALOX5* status utilised in vitro diagnostic multivariate index assays. The benefits of a multivariate index assay were estimated based on the data in both clinical and economic terms (e.g. days off school, days off work for parents, costs of attendance at GP and hospital, and costs of treatment).

Changes in the conduct of the study or planned analyses

No scientifically significant protocol changes occurred during the study. All amendments were approved by the ethics committee unless the sponsor deemed them to be minor amendments. A list of changes is included in *Appendix 6*. No changes in planned analysis occurred after the database was locked.

Discussion of study design

The study design reflects previous work in this area. Short of meta-analysis, an adequately powered, double-blind, placebo-controlled RCT is the gold standard for assessing therapeutic efficacy. The unique aspect of this study was the attempt not only to assess whether or not intermittent montelukast was effective in preschool wheeze, but to prospectively investigate whether or not genetic mutations affecting the metabolism of the cLTs (the endogenous ligand for its target receptor) influenced its efficacy. Previous studies have retrospectively suggested a role for *ALOX5* polymorphisms in LT production, wheeze severity¹³ and montelukast efficacy.^{11,14} However, this is the first study to prospectively test this association. Prospective genetic stratification was necessary to address this pharmacogenetic question.

Study duration

The study was intended to recruit for 24 months. Slower than predicted early recruitment necessitated an increase in recruitment period to 26 months and an expansion of recruitment sites. This extension was approved by the Research Ethics Committee, the regulatory authority and also by the funding body. Thus, recruitment spanned from October 2010 to December 2012 and follow-up was completed in December 2013, with data cleaning, verification and database locking completed by January 2014.

Chapter 3 Results

Recruitment and retention

A total of 1358 subjects were recruited, with 97% in both arms taking part in at least one bimonthly telephone call and thus eligible for inclusion in the primary analysis as per *Figure 3* and *Table 2*.

Protocol deviations

There were 31 reported protocol deviations throughout the study. Very few necessitated withdrawal from the trial, no deviations from protocol exposed a participant to risk of harm, or appeared systematic or particular to an individual site, or had the potential to compromise study validity. Most protocol deviations were addressed by a gentle reminder of the study requirements to the parent or carer. *Table 3* gives details of the study protocol deviations.

Data sets analysed

All analyses were performed on the ITT population (or available case population where outcome data were not available for analysis) unless otherwise stated. These populations are indicated in *Table 4*.

Demographic and other baseline characteristics

Subjects appeared well matched between genotype strata and treatment groups (*Table 5*).

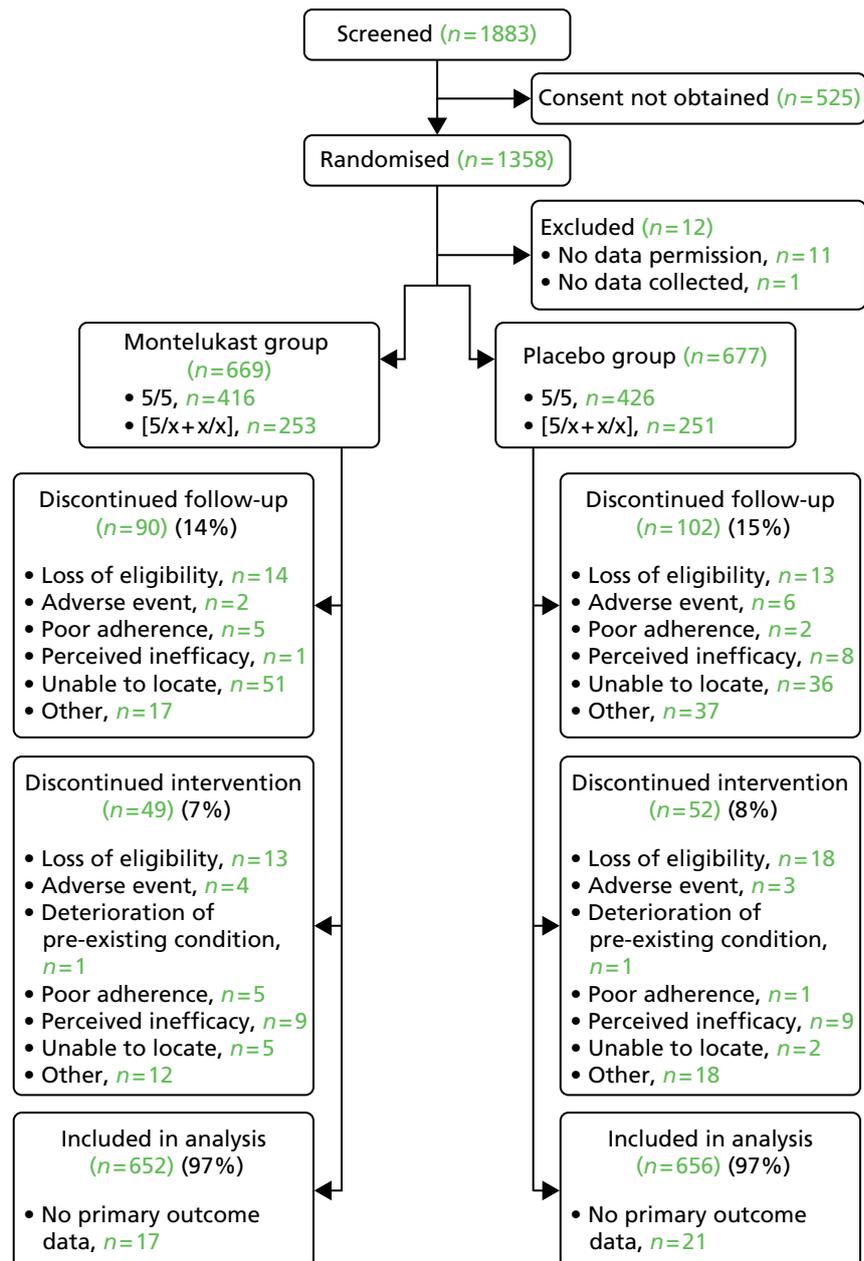


FIGURE 3 The Consolidated Standards of Reporting Trials (CONSORT) diagram.

TABLE 2 Disposition of patients

Patient disposition	Montelukast	Placebo	Total
Enrolled (N)	669	677	1358
Permitted use of data, n (%)	Unknown	Unknown	1346
Received at least one telephone call, n (%)	652 (97)	656 (97)	1308 (96)
Completed 12 months' follow-up, n (%)	579 (87)	575 (85)	1154 (85)
Withdrawn, n (%)	90 (13)	102 (15)	192 (14)
Lost to follow-up, n (%)	51 (8)	36 (5)	87 (6)
AE, n (%)	4 (0.6)	3 (0.4)	7 (0.5)
Death, n (%)	0 (0)	0 (0)	0 (0)
Other, n (%)	37 (6)	60 (9)	97 (7)

TABLE 3 Protocol deviations

Deviation	Recruiting site												
	BR	BD	BI	CA	CO	DE	WH	CH	PO	NO	RO	HG	ST
Entry criteria (n)	0	0	0	0	1	0	0	0	0	0	0	0	0
Withdrawal criteria (n)	0	0	0	0	0	0	0	0	0	0	0	0	0
Concomitant treatment/medication (n)	0	0	0	0	0	0	0	0	0	0	2	0	0
Incorrect dosing regimen (n)	1	0	2	0	2	0	1	0	2	1	4	1	3
Expired medication (n)	1	1	0	0	0	0	0	0	0	0	0	0	0
Incorrect administration (n)	0	0	0	2	0	2	0	0	0	0	0	1	2
Lost samples (n)	0	0	0	0	0	0	2	0	0	0	0	0	0

BD, Bradford; BI, Birmingham; BR, Bristol; CA, Cambridge; CH, Countess of Chester; CO, Coventry; DE, Derby; HG, Harrogate; NO, Nottingham; PO, Portsmouth; RO, Royal Berkshire; ST, University Hospitals of North Staffordshire; WH, Whiston.

TABLE 4 Numbers (%) of individuals withdrawing by month

	Montelukast group	Placebo group
ITT population, n (%)	669 (100)	677 (100)
Timing of last contact		
Withdrew before T0 (no data), n (%)	16 (2)	16 (2)
T0 or earlier (no telephone call data), n (%) ^a	17 (3)	21 (3)
T2 (month 2), n (%)	21 (3)	20 (3)
T4 (month 4), n (%)	15 (2)	12 (2)
T6 (month 6), n (%)	12 (2)	19 (3)
T8 (month 8), n (%)	13 (2)	15 (2)
T10 (month 10), n (%)	12 (2)	15 (2)
T12 (month 12), n (%)	579 (87)	575 (85)
Per-protocol population, n (%)	579 (87)	575 (87)

T2, 2 months after time of first IMP dispensing; T4, 4 months after time of first IMP dispensing; T6, 6 months after time of first IMP dispensing; T8, 8 months after time of first IMP dispensing; T10, 10 months after time of first IMP dispensing; T12, 12 months after time of first IMP dispensing.

a Children withdrawn at T0 or earlier includes those withdrawing before T0; therefore, percentage total is more than 100%.

TABLE 5 Baseline characteristics of study patients

Characteristic	Montelukast group (N = 669)			Placebo group (N = 677)		
	5/5	5/x + x/x	Total	5/5	5/x + x/x	Total
n (%)	416 (62)	253 (38)	669 (100)	426 (63)	251 (37)	677 (100)
Height (cm), mean (SD)	90.0 (10.3)	89.8 (10.5)	89.9 (10.4)	89.9 (10.5)	91.8 (11.7)	90.6 (11.0)
Weight (kg), mean (SD)	14.0 (3.0)	13.9 (3.7)	14.0 (3.3)	14.0 (3.3)	14.6 (3.8)	14.2 (3.5)
Age (years), mean (SD)	2.6 (1.1)	2.5 (1.1)	2.6 (1.1)	2.6 (1.1)	2.8 (1.2)	2.7 (1.1)
Male sex, n (%)	262 (63)	164 (65)	426 (64)	276 (65)	161 (64)	437 (65)
Ethnic origin						
White, n (%)	335 (81)	179 (71)	514 (77)	338 (79)	174 (69)	512 (76)
Black, n (%)	5 (1)	14 (6)	19 (3)	4 (1)	14 (6)	18 (3)
Asian, n (%)	55 (13)	37 (15)	92 (14)	58 (14)	46 (18)	104 (15)
Other, n (%)	21 (5)	23 (9)	44 (7)	26 (6)	17 (7)	43 (6)
Preterm birth (< 37 weeks), n (%)	58 (14)	40 (16)	98 (14)	56 (13)	42 (17)	98 (15)
Birthweight (< 2500 g), n (%)	51 (12)	28 (11)	79 (12)	42 (10)	28 (11)	70 (10)
Food allergy, n (%)	64 (15)	44 (18)	108 (16)	64 (15)	47 (19)	111 (17)
Drug allergy, n (%)	26 (6)	12 (5)	38 (6)	23 (6)	19 (8)	42 (6)
Itchy rash (> 6 months, ever), ^a n (%)	98 (23)	64 (25)	162 (24)	104 (25)	60 (24)	164 (25)
Eczema (ever), ^b n (%)	207 (49)	121 (48)	328 (48)	215 (52)	134 (53)	349 (52)
History of asthma in mother, n (%)	156 (37)	95 (38)	251 (37)	141 (34)	89 (35)	230 (34)
History of asthma in father, n (%)	126 (30)	73 (29)	199 (29)	126 (30)	81 (32)	207 (31)
Age at first wheeze (months), mean (SD)	12.4 (9.8)	13.5 (10.5)	12.8 (10.1)	12.4 (10.4)	13.6 (11.5)	12.9 (10.8)
Children with episodic viral wheeze, n (%)	296 (71)	181 (72)	477 (71)	295 (69)	191 (76)	486 (72)
Children with multitrigger wheeze, n (%)	120 (29)	72 (28)	192 (29)	131 (31)	60 (24)	191 (28)
Interval between onset of URTI and wheezing (hours), ^c mean (SD)	31.6 (27.4)	28.8 (25.2)	30.5 (26.6)	27.3 (23.4)	28.2 (26.0)	27.7 (24.4)
Children with more than one hospital admission for wheeze in the past year, n (%)	363 (87)	216 (85)	579 (87)	351 (82)	203 (81)	554 (82)
Courses of oral corticosteroids in past year, mean (SD)	2.0 (1.9)	1.8 (1.8)	1.9 (1.8)	1.9 (1.9)	1.8 (2.0)	1.9 (2.0)
USMA in previous year, mean (SD)	5.5 (4.3)	5.4 (4.1)	5.4 (4.2)	5.7 (5.3)	5.6 (4.6)	5.6 (5.1)
Continuous inhaled corticosteroids, n (%)	118 (28)	66 (26)	184 (28)	144 (34)	69 (27)	213 (31)

URT, upper respiratory tract infection; USMA, unscheduled medial attendance for wheeze.

a A question to parents from the International Study of Asthma and Allergies in Childhood questionnaire was used to identify symptoms suggestive of eczema.

b Eczema from birth was based on a parental report to the recruiting investigator at enrolment.

c Based on a parental report of the usual interval between a URTI and the onset of wheezing.

Assessment of treatment compliance

To assess compliance, patient carers were asked to return empty/unused/expired sachets to the sponsor in self-addressed prepaid envelopes; however, returns were too low to provide any meaningful data.

Efficacy results and tabulations of patient data

Primary outcome

There was no difference between the montelukast and placebo group for the primary outcome (Table 6).

Secondary outcomes

A possible effect was seen within the 5/5 genotype stratum (Figure 4), with a suggestion of increased responsiveness in this group.

TABLE 6 Effect size and CI for primary outcome

Outcome measure	Montelukast group	Placebo group	Adjusted IRR (95% CI)	p-value
Analysis population, n (%)	652 (50)	656 (50%)		
Unscheduled medical attendance for wheeze episodes, mean (SD)	2.0 (2.6)	2.3 (2.7)	0.88 (0.77 to 1.01)	0.06

Data were analysed using Poisson regression with fixed effects for stratification factor and treatment group, and a random effect for individuals to account for overdispersion, with follow-up time fitted as the exposure. Follow-up time is based on time from randomisation until either the 12-month end of trial date or date of last telephone call. Primary outcome data were taken from the telephone call, which occurred every 2 months. Children were included in the analysis if they had at least one telephone call recorded and a follow-up time was then fitted as an exposure in the model.

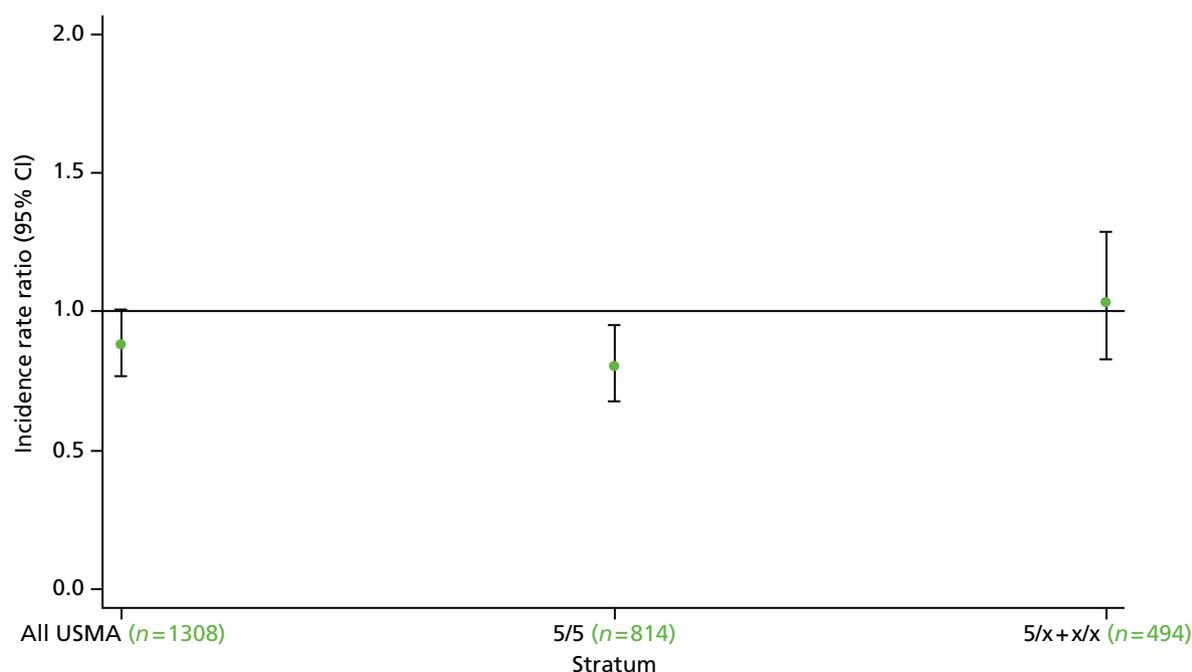


FIGURE 4 Forest plot of unscheduled medical attendances by genotype stratum. USMA, unscheduled medical attendance.

Statistical/analytical issues

The study was limited in that, although adequately powered to address the efficacy of intermittent montelukast in preschool wheeze, it had the power to detect only a rather substantial interaction between genotype and efficacy. Thus, the suggestion ($p = 0.01$) of improved efficacy in the 5/5 stratum is not mathematically robust when exposed to a test for interaction ($p = 0.08$; *Table 7*). The interquartile range for the time to first unscheduled medical attendance (USMA) was not calculable, as more than 25% of children never required an USMA.

There was no apparent influence of wheeze phenotype, use of inhaled steroids at baseline or alternative genotype stratum on USMA (*Table 8*).

There was an increased time to first USMA requiring hospital admission for wheeze in the montelukast group (but not for other types of USMA) and a decreased use of rescue oral corticosteroids (*Table 9*).

TABLE 7 Subgroup analysis of treatment response in the 5/5 and [5/x + x/x] strata

Outcome measure	Montelukast group	Placebo group	Adjusted IRR (95% CI)	p-value	p-value (interaction)
USMA in the 5/5 stratum, mean (SD)	2.0 (2.7)	2.4 (3.0)	0.80 (0.68 to 0.95)	0.01	0.08
USMA in the [5/x + x/x] stratum, mean (SD)	2.0 (2.5)	2.0 (2.3)	1.03 (0.83 to 1.29)	0.79	

TABLE 8 Other prespecified subgroup analyses of treatment response

Outcome measure	Montelukast group, mean (SD)	Placebo group, mean (SD)	Adjusted IRR (95% CI)	p-value	p-value (interaction)
USMA in the 5/5 + 5/x stratum	2.0 (2.6)	2.3 (2.8)	0.88 (0.76 to 1.00)	0.06	0.93
USMA in the x/x stratum	1.7 (1.8)	1.9 (2.0)	0.85 (0.44 to 1.66)	0.64	
Inhaled corticosteroid use at baseline	2.0 (3.0)	2.0 (2.3)	1.01 (0.82 to 1.24)	0.92	0.09
No inhaled corticosteroid use at baseline	2.0 (2.2)	2.5 (3.0)	0.80 (0.67 to 0.96)	0.01	
Multitrigger wheeze ^a	2.1 (3.0)	2.0 (2.5)	1.01 (0.79 to 1.31)	0.90	0.19
Episodic viral wheeze ^b	2.0 (2.4)	2.3 (2.9)	0.83 (0.71 to 0.97)	0.03	

a Phenotype where wheeze can occur in absence of a viral cold.

b Phenotype characterised by wheeze occurring only in the context of a viral cold.

TABLE 9 Secondary outcomes

Outcome measure	Montelukast group	Placebo group	Adjusted IRR, OR or HR (95% CI)	p-value
Children with one or more USMAs, <i>n</i> (%)	426 (65)	456 (70)	OR 0.83 (0.66 to 1.04)	0.10
Time (days) to first USMA, median (interquartile range) ^a	147 (50–365)	130 (38–) ^b	HR 0.89 (0.78 to 1.02)	0.09
Need for rescue oral steroids (courses per child), mean (SD) ^c	0.26 (0.7)	0.33 (0.9)	IRR 0.75 (0.58 to 0.98)	0.03
Wheeze episodes, mean (SD) ^c	2.7 (2.9)	2.6 (3.0)	IRR 1.02 (0.91 to 1.16)	0.68
Duration (days) of wheeze episodes, mean (SD)	5.2 (4.0)	5.4 (3.9)	IRR 0.97 (0.89 to 1.06)	0.53
Duration (days per child) of hospital admission, mean (SD) ^d	1.8 (1.3)	1.7 (1.1)	IRR 1.05 (0.94 to 1.18)	0.40
Symptomatic days per wheeze episode, mean (SD)	4.9 (3.5)	4.8 (3.8)	IRR 0.96 (0.88 to 1.05)	0.36

HR, hazard ratio; OR, odds ratio.

a Seven participants were missing dates for USMAs and seven participants had their first medical attendance on the day of randomisation and are hence excluded. Time to first USMA data were analysed using a Cox regression model with fixed effects for stratification factor and treatment group.

b The 75th percentile could not be calculated for this interquartile range because more than 25% of children had no USMAs.

c Analysis included all children. A total of 446 children had no diary data and these were considered to have no wheeze and cold episodes. The analysis was repeated treating these patients as missing and there was no difference in the IRR between treatment and placebo groups.

d The duration of each hospital admission was analysed using Poisson regression with fixed effects for stratification factor and treatment group, a random effect for individuals, with follow-up time fitted as the exposure.

Note

Data were analysed using Poisson regression with fixed effects for stratification factor and treatment group, a random effect for individuals to account for overdispersion, with follow-up time fitted as the exposure. Follow-up time is based on time from randomisation until either the 12-month end of trial date or date of last telephone call. An interaction term was included to assess whether or not there was a differential treatment effect dependent on genetic stratum.

Chapter 4 Safety evaluation

Adverse events

Table 10 shows AEs reported during the duration of the trial. Section A shows a breakdown by intensity, followed by category (B) for all AEs. Subsequent sections (C–G) reflect the likelihood, as assessed by the (blinded) local PI, that the AE was attributable to the trial drug. Of the 940 AEs reported in the study, 657 (70%) were classified as definitely not related to study drug, 179 (19%) as probably not related, 93 (10%) as possibly related, 11 (1%) as probably related and no AE was definitely related. We recorded one SAE, which was a skin reaction in a child allocated to placebo. The distribution of AEs was similar between treatment groups. There were no recorded deaths.

Safety conclusions

This study supports the position that montelukast is safe in this age group. No excess of AEs was observed in the treatment group, nor were any novel AEs identified over and above those known prior to study commencement.

TABLE 10 Adverse events

	Montelukast group (N = 669)	Placebo group (N = 677)	Total (N = 1346)
Event numbers	n (%)	n (%)	n (%)
Total number of events	397 (100)	543 (100)	940 (100)
Total number of participants	197 (29)	235 (35)	432 (32)
(A) Intensity	397	543	940
Mild	314 (79)	426 (78)	740 (79)
Moderate	77 (19)	108 (20)	185 (20)
Severe	6 (2)	9 (2)	15 (2)
(B) Category	397	543	940
Minor injury	27 (7)	22 (4)	49 (5)
Gastrointestinal	86 (22)	122 (22)	208 (22)
Upper respiratory tract infection	73 (18)	103 (19)	176 (19)
Central nervous system	25 (6)	46 (8)	71 (8)
Minor infection	87 (22)	107 (20)	194 (21)
Allergy	16 (4)	20 (4)	36 (4)
Cutaneous	32 (8)	54 (10)	86 (9)
Respiratory	34 (9)	54 (10)	88 (9)
Haematological	5 (1)	7 (1)	12 (1)
Genitourinary	10 (3)	6 (1)	16 (2)
Major injury	2 (1)	1 (< 1)	3 (< 1)
Musculoskeletal	0 (0)	1 (< 1)	1 (< 1)

continued

TABLE 10 Adverse events (continued)

Event numbers	Montelukast group (N = 669) n (%)	Placebo group (N = 677) n (%)	Total (N = 1346) n (%)
(C) Total number of events: definitely not related	281	376	657
Minor injury	27 (10)	22 (6)	49 (7)
Gastrointestinal	40 (14)	62 (16)	102 (16)
Upper respiratory tract infection	63 (22)	88 (23)	151 (23)
Central nervous system	8 (3)	10 (3)	18 (3)
Minor infection	76 (27)	91 (24)	167 (25)
Allergy	13 (5)	16 (4)	29 (4)
Cutaneous	18 (6)	32 (9)	50 (8)
Respiratory	25 (9)	47 (13)	72 (11)
Haematological	2 (1)	2 (1)	4 (1)
Genitourinary	7 (2)	4 (1)	11 (2)
Major injury	2 (1)	1 (< 1)	3 (< 1)
Musculoskeletal	0 (0)	1 (< 1)	1 (< 1)
(D) Total number of events: probably not related	80	99	179
Minor injury	0 (0)	0 (0)	0 (0)
Gastrointestinal	26 (33)	33 (33)	59 (33)
Upper respiratory tract infection	10 (13)	15 (15)	25 (14)
Central nervous system	5 (6)	8 (8)	13 (7)
Minor infection	11 (14)	16 (16)	27 (15)
Allergy	3 (4)	4 (4)	7 (4)
Cutaneous	10 (13)	13 (13)	23 (13)
Respiratory	9 (11)	7 (7)	16 (9)
Haematological	3 (4)	1 (1)	4 (2)
Genitourinary	3 (4)	2 (2)	5 (3)
Major injury	0 (0)	0 (0)	0 (0)
Musculoskeletal	0 (0)	0 (0)	0 (0)

TABLE 10 Adverse events (continued)

Event numbers	Montelukast group (N = 669) n (%)	Placebo group (N = 677) n (%)	Total (N = 1346) n (%)
(E) Total number of events: possibly related	33	60	93
Minor injury	0 (0)	0 (0)	0 (0)
Gastrointestinal	19 (58)	23 (38)	42 (45)
Upper respiratory tract infection	0 (0)	0 (0)	0 (0)
Central nervous system	10 (30)	25 (42)	35 (38)
Minor infection	0 (0)	0 (0)	0 (0)
Allergy	0 (0)	0 (0)	0 (0)
Cutaneous	4 (12)	8 (13)	12 (13)
Respiratory	0 (0)	0 (0)	0 (0)
Haematological	0 (0)	4 (7)	4 (4)
Genitourinary	0 (0)	0 (0)	0 (0)
Major injury	0 (0)	0 (0)	0 (0)
Musculoskeletal	0 (0)	0 (0)	0 (0)
(F) Total number of events: probably related	3	8	11
Minor injury	0 (0)	0 (0)	0 (0)
Gastrointestinal	1 (33)	4 (50)	5 (45)
Upper respiratory tract infection	0 (0)	0 (0)	0 (0)
Central nervous system	2 (67)	3 (38)	5 (45)
Minor infection	0 (0)	0 (0)	0 (0)
Allergy	0 (0)	0 (0)	0 (0)
Cutaneous	0 (0)	1 (13)	1 (9)
Respiratory	0 (0)	0 (0)	0 (0)
Haematological	0 (0)	0 (0)	0 (0)
Genitourinary	0 (0)	0 (0)	0 (0)
Major injury	0 (0)	0 (0)	0 (0)
Musculoskeletal	0 (0)	0 (0)	0 (0)
(G) Total number of events: definitely related	0	0	0
Note Percentages correspond to the totals of each section, rather than the overall total. Percentages may not total to 100 because of rounding.			

Chapter 5 Clinical laboratory evaluation

Urinary eicosanoids

Urinary eicosanoids were evaluated at baseline and, in a subset of recruits, during exacerbation of wheeze. The numbers providing paired (baseline and exacerbation of wheeze) urine samples were insufficient to justify detailed analysis; however, samples are stored for further analysis and data remain available for future use. Baseline urine was analysed by genotype stratum (*Figure 5*). There was a statistically significant increase in baseline level of LT activation in subjects with no wild-type (5 repeat) *ALOX5* promoter allele. This is contrary to the direction predicted from the non-significant genotype–efficacy interaction suggested in *Table 7*. The numbers in the x/x group are very small; thus, this observation must be treated with caution.

Genetics

Arachidonate 5-lipoxygenase genotype was compared with self-reported ethnicity (*Table 11*). There was marked genotypic variation between ethnicities, with black subjects having a lower frequency of 5/5 alleles than whites or Asians, and also having the highest frequency of x/x alleles. A clinical correlation has not been established.

Exploratory SNP analysis is under way and will form the basis of a future manuscript. Consent exists for future related genetic analyses to be performed by this team and others.

Concomitant medication use

Subjects were permitted to use any concomitant medications excluding LT receptor antagonists. A record was kept of concomitant medication usage. There was no difference in reported salbutamol usage between treatment groups. A statistically significant reduction in oral corticosteroid usage was observed ($p = 0.03$; see *Table 9*).

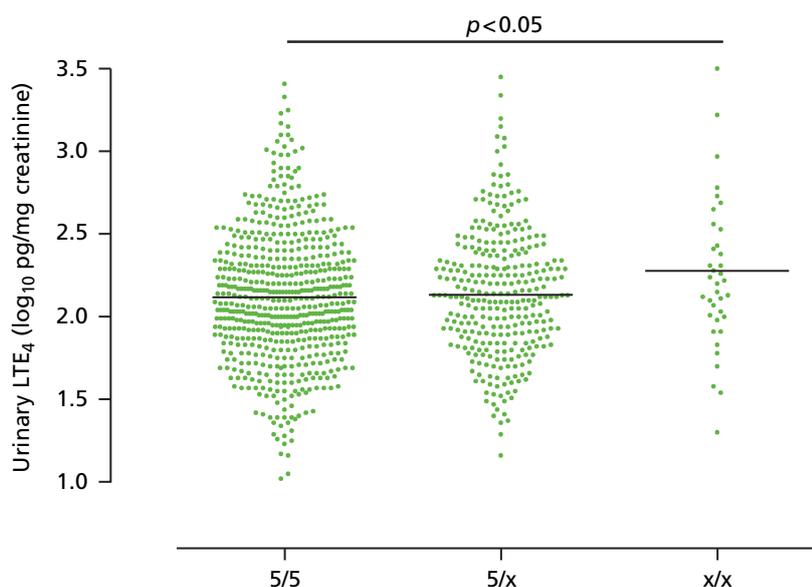


FIGURE 5 Urinary LTE₄ levels by *ALOX5* promoter genotype.

TABLE 11 Distribution of *ALOX5* promoter polymorphism genotype by parent-reported ethnicity

Genotype	White, n (%)	Black, n (%)	Asian, n (%)	Bangladeshi, n (%)	Mixed, n (%)	Other, n (%)	All, n (%)
3/3	0 (0.00)	4 (10.81)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	4 (0.29)
3/4	1 (0.10)	2 (5.41)	0 (0.00)	1 (0.75)	2 (2.78)	1 (2.70)	7 (0.51)
3/5	4 (0.39)	6 (16.22)	0 (0.00)	0 (0.00)	10 (13.89)	4 (10.81)	24 (1.76)
3/6	0 (0.00)	2 (5.41)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.15)
3/7	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (1.39)	1 (2.70)	2 (0.15)
4/4	18 (1.75)	0 (0.00)	8 (13.34)	5 (3.73)	2 (2.78)	1 (2.70)	34 (2.49)
4/5	285 (27.78)	10 (27.03)	18 (30)	33 (24.63)	11 (15.28)	7 (18.92)	364 (26.65)
4/6	6 (0.58)	0 (0.00)	1 (1.67)	2 (1.49)	0 (0.00)	1 (2.70)	10 (0.73)
5/5	677 (65.98)	9 (24.32)	27 (45)	83 (61.94)	43 (59.72)	19 (51.35)	858 (62.81)
5/6	30 (2.92)	4 (10.81)	5 (8.33)	10 (7.46)	3 (4.17)	2 (5.41)	54 (3.95)
6/6	0 (0.00)	0 (0.00)	1 (1.67)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
2/4	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
5/8	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
5/7	2 (0.19)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	2 (0.15)
2/5	1 (0.10)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (0.07)
3/8	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	1 (2.70)	1 (0.07)
Total	1026 (100)	37 (100)	60 (100)	134 (100)	72 (100)	37 (100)	1366 ^a (100)
5/5	677 (65.98)	9 (24.32)	27 (45.00)	83 (61.94)	43 (59.72)	19 (51.35)	858 (62.81)
5/X	323 (31.48)	20 (54.05)	23 (38.33)	43 (32.09)	24 (33.33)	13 (35.14)	446 (32.65)
X/X	26 (0.19)	8 (21.62)	10 (16.67)	8 (5.97)	5 (6.94)	5 (13.51)	62 (4.54)
Total	1026 (100)	37 (100)	60 (100)	134 (100)	72 (100)	37 (100)	1366 ^a (100)

a A total of 1366 patients gave consent and were genotyped. Of these, 8 withdrew prior to randomisation and 12 were subsequently excluded from the analysis owing to parental withdrawal of permission to use data.

Qualitative study outcomes

The parents of 42 subjects agreed to a qualitative interview; a sizeable proportion of these were of Bangladeshi origin. Interview design is detailed elsewhere,¹⁵ but where necessary a Bangladeshi translator was available. From this study, Bangladeshi families appear particularly motivated to participate in clinical trials, despite their understanding of study concepts being limited by educational attainment or language. The decision to participate was driven primarily by rapport with the researcher, with quality of study literature being of less importance. Where a study population has a Bangladeshi (or perhaps south Asian) bias, particular emphasis should be placed on face-to-face verbal explanation of trial concepts and procedures. Further detail regarding qualitative study outcomes is beyond the scope of this report and the article is available via Open Access Online.¹⁵

Health economic outcomes

The health economic analysis was dependent upon a demonstrable treatment effect. In the absence of a treatment effect of montelukast, further analysis was deemed unwarranted.

Chapter 6 Discussion and overall conclusions

This study is, overall, negative with regard to the primary outcome, indicating no benefit from intermittent montelukast treatment in preschool children with wheeze. This supports the recent findings of Valovirta *et al.*,¹⁶ who compared intermittent and regular montelukast with placebo and found no benefit. There was an increased time to first USMA requiring hospital admission for wheeze in the montelukast group (but not for other types of USMA) and an increased use of rescue oral corticosteroids; however, the study was not powered to demonstrate these effects and the patchiness of the effect makes its validity questionable. There was no apparent influence of wheeze phenotype, use of inhaled steroids at baseline or alternative genotype stratum on USMA, although wheeze phenotype was based on parental reporting and mean daily dose of inhaled steroids was not assessed. The IRR seen in the montelukast group compared with placebo was 0.88 ($p = 0.06$) in favour of montelukast, not meeting statistical significance. A larger trial might have power to identify a difference of this magnitude, but the clinical benefit may not justify the exercise; this should be considered in the design of future studies.

A possible effect was seen within the 5/5 genotype stratum, with a suggestion of increased responsiveness in this group (contrary to Lima's *et al.* finding,¹¹ but consistent with Telleria *et al.*¹⁴), but the test for genotype–efficacy interaction was not confirmatory. Furthermore, the small effect seen in urinary LTE₄ levels at baseline was not supportive. Future work will prospectively study montelukast efficacy in the 5/5 genotype stratum and explore the role of putative response modifiers like environmental tobacco smoke exposure¹⁷ and air pollution.

The search for an effective therapy for preschool wheezing illness is hampered by the lack of a clearly defined phenotype with robust biomarkers. This study adopted a pragmatic approach, recruiting a heterogeneous population encompassing numerous likely aetiologies, in the hope that inhibition of LT activity might address a mechanistic pathway common to these probably distinct but overlapping clinical entities. There is evidence to implicate cLTs in a proportion of preschool wheezing disease^{5,12} and a greater success in assessing LTE₄ levels during wheeze exacerbation (as opposed to at baseline) might have shed light on the value of this hypothesis and thus the viability of montelukast as a therapeutic target. The lack of a clear genotype–urinary LTE₄ level correlation may reflect a lower than anticipated importance of *ALOX5* promoter polymorphism genotype or perhaps that the differences become more evident during exacerbation compared with during convalescence. The LT pathway is complex, and it is possible that several mutations (perhaps in combination, perhaps with an epigenetic influence¹⁷) play a more important role in determining LT activity and montelukast response in this population than *ALOX5*. Future work will investigate the role of other genes on LTE₄ output and montelukast response, and consideration should be given to stratification of montelukast response trials by LTE₄ levels measured during wheeze exacerbation (or perhaps following a standardised challenge).

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TABLE 12 Principal study personnel

Title	Name	Affiliation
Chief investigator	Professor Jonathan Grigg	Queen Mary University of London
Co-ordinating PI	Dr Chinedu Nwokoro	Queen Mary University of London
PI	Professor Chris Griffiths	Queen Mary University of London
	Dr Steve Turner	Royal Aberdeen Children's Hospital
	Dr Hitesh Pandya	University Hospitals Leicester
See <i>Acknowledgements</i> for other PIs		
Statistics team	Dr Sandra Eldridge	Queen Mary University of London
	Miss Clare Rutterford	Queen Mary University of London
Independent Trial Steering Committee	Professor Warren Lenney	University Hospitals, North Staffordshire
	Professor David Price	University of Aberdeen
	Dr Jay Panickar	Royal Manchester Children's Hospital
	Dr Hussain Mulla	University Hospitals Leicester
	Dr Edward Simmonds	Walsgrave General Hospital
	Professor Robert Walton	Queen Mary University of London
	Professor John Holloway	University of Southampton
DSMC	Professor Andy Bush (chairperson)	Royal Brompton Hospital
	Paul Lambert (statistician)	University of Leicester
	Ian Jarrold	British Lung Foundation
Sponsor	Mr Gerry Leonard	Queen Mary University of London
Project managers	Ms Suzi Miranbeg	Queen Mary University of London
	Miss Cassie Brady	Queen Mary University of London
Research nurses	Mrs Teresa McNally	University Hospitals Leicester
	Ms Belinda Howell	Queen Mary University of London
	Ms Donna Nelson	Royal Aberdeen Children's Hospital
Laboratory investigator	Dr Tom Vulliamy (Lead)	Queen Mary University of London
	Mr Iain Dickson	Queen Mary University of London
	Ms Lee Koh	Queen Mary University of London
	Professor Marek Sanak	Jagellonian University, Krakow
Data management	Miss Hafiza Khatun	Queen Mary University of London
	Miss Sandy Smith	Queen Mary University of London

Data and Safety Monitoring Committee: Professor Andy Bush (chairperson), Professor Paul Lambert (statistician) and Ian Jarrold (Head of Research, British Lung Foundation).

Independent members of the Trial Steering Committee: Professor Warren Lenney (chairperson) and Dr Edward Simmonds.

Trial Support Group: Ms Catherine Brady, Ms Amy Hoon (data monitoring), Ms Donna Nelson, Ms Bea Howell, Ms Theresa McNally (multicentre co-ordination), Ms Hafiza Khatun (data audit), Ms Suzi Miranbeg (trial co-ordination), Mr Gordon Forbes (statistics), Ms Mandy Wan and Ms Nanna Christiansen (pharmacy).

Local investigators in secondary care centres: Dr Christopher Upton (Norfolk and Norwich University Hospitals NHS Trust), Dr Maria O’Callaghan (Barts Health NHS Trust, Whipps Cross Hospital), Dr S Murthy Saladi (Countess of Chester NHS Foundation Trust), Dr Catherine Tuffrey (Portsmouth Hospitals NHS Trust), Dr Sheng-Ang Ho (East Cheshire NHS Trust), Dr Robert Ross Russell (Cambridge University Hospitals NHS Trust), Dr Anil Tuladhar (North Tees and Hartlepool NHS Trust), Dr Edwin Osakwe (Oxford Radcliffe Hospitals NHS Trust), Dr Paul McNamara (Alder Hey Children’s NHS Trust), Dr James Y Paton (NHS Lothian University Hospitals, Royal Hospital for Sick Children), Dr Mansoor Ahmed (Burton Hospitals NHS Foundation Trust), Dr John Alexander (University Hospital of North Staffordshire NHS Trust), Dr Deepthi Jyothish (Birmingham Children’s Hospital NHS Trust), Dr John Scanlon (Worcestershire Acute NHS Trust), Dr Edward Simmonds (University Hospitals of Coventry NHS Trust), Dr James Crossley (Chesterfield Royal NHS Foundation Trust), Dr Shakeel Rahman (Harrogate and District NHS Foundation Trust), Professor Harish Vyas (Nottingham University Hospitals NHS Trust), Dr Will Carroll (Royal Derby Hospitals NHS Trust), Dr Diarmuid P Kerrin (Barnsley NHS Foundation Trust), Dr Hazel Evans (Southampton University Hospitals NHS Trust), Dr Anna Mathew (Western Sussex NHS Hospitals Trust), Dr Anne Prendiville (Royal Cornwall Hospital Trust), Professor Mark Everard (Sheffield Children’s NHS Foundation Trust), Dr Lakshmi Chilukuri (St Helens and Knowsley Teaching Hospitals NHS Trust), Dr Sharryn Gardner (Southport and Ormskirk NHS Trust), Dr Gary Ruiz (King’s College Hospital Foundation NHS Trust), Dr Simon Langton Hewer (University Hospitals Bristol NHS Trust), Dr Peter DeHalpert (Royal Berkshire NHS Foundation Trust), Dr Paul Seddon (Brighton and Sussex University Hospitals NHS Trust), Dr Tim Adams (NHS Ayrshire and Arran), Dr David Cremonesini (Hinchingbrooke Health Care NHS Trust), Dr Jonathan Garside (Calderdale and Huddersfield NHS Trust), Dr Anil Shenoy (Bradford Teaching Hospitals NHS Trust), Dr Matthew Babirecki (Airedale NHS Foundation Trust), Dr Anne Ingram (Luton and Dunstable Hospital NHS Trust), Dr John Furness (County Durham and Darlington NHS Trust), Dr David Lacy (Wirral University Teaching Hospital NHS Trust) and Dr Mike Linney (Western Sussex Hospitals NHS Trust).

Primary care recruitment: Springfield GP-led Health Centre, Lower Clapton Practice, The Lawson Practice, Neaman Practice, Elm Practice, Sandringham Practice, Queensbridge Group Practice, Latimer Health Centre, Statham Grove Surgery. In Tower Hamlets Primary Care Trust: Strouts Place Medical Centre, Jubilee Street Practice, Wapping Health Centre, East One Health, Barkantine Health Centre, Bliethehale Health Centre, Albion Health Centre, Chrisp Street Practice, Bromley-By-Bow Health Centre, XX Place Surgery, St Andrews Health Centre and Mission Practice.

Finally, we thank our participants and their caregivers.

Contributions of authors

Chinedu Nwokoro supervised the study, and wrote with **Jonathan Grigg** the first and final drafts of the report.

Hitesh Pandya, Stephen Turner, Christopher J Griffiths and **David Price** contributed to study planning and to the final manuscript.

Sandra Eldridge contributed to study planning and supervised the statistical analysis.

Tom Vulliamy contributed to study planning, supervised genotype analysis and contributed to the final manuscript.

Marek Sanak performed the urinary LT analysis and contributed to the final manuscript.

John W Holloway contributed to study planning, advised on genotype analysis and contributed to the final manuscript.

Rossa Brugha supervised the study, did the combined analysis and contributed to the final manuscript.

Lee Koh performed genotyping and was responsible for the audit of genotype data.

Iain Dickson contributed to study planning, genotype analysis and the final manuscript.

Clare Rutterford supported the data monitoring committee, wrote the final statistical analysis plan and did the statistical analysis.

Jonathan Grigg was the chief investigator, planned and provided overall supervision of the study, wrote with **Chinedu Nwokoro** the first and final drafts of the report and vouches for these data.

Publications

MacNeill V, Nwokoro C, Griffiths C, Grigg J, Seale C. Recruiting ethnic minority participants to a clinical trial: a qualitative study. *BMJ Open* 2013;**3**:e002750.¹⁵

Nwokoro C, Pandya H, Turner S, Eldridge S, Griffiths CJ, Vulliamy T, *et al.* Intermittent montelukast in children aged 10 months to 5 years with wheeze (WAIT trial): a multicentre, randomised, placebo-controlled trial. *Lancet Respir Med* 2014;**2**:796–803.¹⁸

Conference abstracts

Brady C. Delivering WAIT Across Multiple Settings. Royal College of Paediatrics and Child Health Annual Meeting, Birmingham, UK, 28–30 April 2015.

Grigg J. The WAIT Study of Parent-determined Oral Montelukast Therapy for Pre-school Wheeze – Introduction to the Study, Key Findings, and Plans for Further Research. Royal College of Paediatrics and Child Health Annual Meeting, Birmingham, UK, 28–30 April 2015.

Nwokoro C. The Wheeze and Intermittent Treatment (WAIT) Trial: Results and What Next? John Price Respiratory Conference, London, UK, 24–25 March 2015.

Nwokoro C. WAIT – Working Together to Deliver a Large Paediatric Trial. Royal College of Paediatrics and Child Health Annual Meeting, Glasgow, UK, 5–8 June 2013.

Nwokoro C. Recruiting Ethnic Minority Participants to a Clinical trial: Qualitative Study. Royal College of Paediatrics and Child Health Annual Meeting, Glasgow, UK, 5–8 June 2013.

Nwokoro C. Lipid Mediators in Preschool Wheezing Disorders. Barts and the London School of Medicine and Dentistry. Paediatric Research Seminar. London, UK 14 February 2013.

Nwokoro C. Urinary Eicosanoids and Preschool Wheeze Phenotype. European Respiratory Society Annual Congress, Vienna, Austria, 1–5 September 2012.

Data sharing statement

All available data can be obtained from the corresponding author.

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Appendix 1 Patient information sheet

Centre Name: Royal London Hospital

Centre Number: _____ (as appropriate)

The “WAIT” Study; Wheeze And Intermittent Treatment

We are inviting parents¹ and their children to take part in a research study. Before you decide if you would like to take part it is important that you understand why the research is being done and what it will mean for you and your child. This information sheet gives all of the important information about our trial. We have divided this information sheet into two parts:

Part One

Tells you the purpose of the research and what will happen if you decide to take part.

Part Two

Gives you more detailed information about how the study will be organised.

Part 1

What is the purpose of the study?

Attacks of wheeze (the noise we make when our airways become narrowed) are very common in children under 6 years of age (we call this preschool wheeze). Most of these attacks happen during colds, but in some children wheeze can happen between colds as well. We know that most young children grow out of their wheeze after the age of 6 years. At the moment we don't know the best way to stop these attacks of wheeze but we think that a medicine called “Montelukast” will make the attacks of wheeze less severe. Montelukast stops a substance in the body called “leukotriene” from narrowing the airways and causing wheeze. It is already licensed as safe for young children – but at the moment is used only as an “add on” to regular inhaled steroids and it has to be given every day.

We think that Montelukast may be helpful in preschool wheeze on its own, and that regular daily use may not be necessary. We have designed this study to see if Montelukast, if started by parents at the first sign of a cold and stopped when children are better, may prevent wheeze becoming so bad that your child needs to

¹ Where we use the word 'Parent' we mean people who have parental responsibility, which may include a legal representative (guardian).

see your GP or emergency doctor. To see if montelukast really works in the way we think we have to give some children the active medicine – montelukast coated onto granules of sugar taken by mouth once a day - and some children the sugar granules only (with no montelukast). No one knows in advance which one your child will get. This sort of study is called a “randomised controlled trial”.

Studies of montelukast in adults with asthma have shown that genetic make-up affects whether someone responds very well, or not so well to montelukast. Another aim of our trial is to measure the amount of leukotriene produced by the body and the genes that control it to see if we can identify children who may respond very well to montelukast.

What is the drug, device or procedure that is being tested?

The medicine that we are testing is called montelukast. Its “trade name” is “Singulair”. Montelukast is not a new medicine and has been licensed as safe for use in young children for several years. It comes as granules in individually packaged sachets and can be given either directly into the mouth, or mixed with a spoonful of cold or room temperature soft food (e.g. apple sauce, ice cream, carrots or rice). The granules consist of a sugar core with a fine coating of the drug. Each dose of montelukast granules stops the airway narrowing effect of leukotriene for 24 hours – so you only need to give it once a day. Some children will be given the core sugar granules, but without the coating of montelukast, this is called a placebo medicine and has no effect. These are packed so they look and taste exactly the same as montelukast granules.

Why has my child been chosen?

Your child has been asked to take part in this study because he/she has had at least 2 episodes of wheeze. Your General Practitioner (GP), specialist asthma nurse or hospital doctor thinks your child might be suitable to take part in this study and wants to refer them to the research team to assess this. We will be recruiting 1300 children for this study from a number of children’s hospitals across the UK as well as from GP practices.

Does my child have to take part?

No, taking part is completely voluntary. It is up to you to decide whether or not to take part. Even if you do agree to join, you can drop out at any time without giving a reason. A decision to leave the study, or a decision not to take part, will not in any way affect the standard of care you and your child receive now or in the future. If you

change your mind about staying in the study we would appreciate it if you would let us know. The study doctor may also stop your child from taking the study treatments at any time if they feel it is best for them to do so. However, if this happens, they will still want to carry on collecting information from your child if you both agree.

What will happen to my child if we agree to take part and how long will it take?

If you do take part, you will be given this information sheet to keep and be asked to sign a consent form. We would like your child to remain in the study for a year. If you agree to take part, you will have another visit to receive the medication. After that we will be contacting you by phone or email only. We may ask some of you to allow us a more extended interview about parents' views on the study and if so we will visit your home at the end of the study. Each visit will last under an hour. We will now explain what will happen at each of the visits.

Visit 1².

If you are interested in taking part, and are satisfied with the explanations from your research team, you will be asked to sign a consent form at your first clinic visit. You will be given a copy of this information sheet and your signed consent forms to keep. Once consent has been given, you and your child will be asked some questions to make sure that they are suitable to join. The research doctor or nurse will want to know about your child's wheezing symptoms. They will ask some questions about your child's medical history and what other medicines they are taking. We will check that you can use the salbutamol (blue) inhaler properly so that they are getting the right amount of medication each time they use it. The doctor or nurse will also collect a saliva sample from your child using a specially designed mini-sponge which is entirely painless. The saliva will be analysed for genes (DNA) for leukotrienes. We will give you a container to collect some urine on the day of visit number 2. We will measure the amount of leukotrienes in your child's urine.

The amount of leukotriene in our urine can be affected by exposure to tobacco smoke (this can come from the air breathed out by smokers nearby – it does not mean that you or your child is a smoker) and so we will also measure levels of cotinine (produced if we are exposed to tobacco smoke) in the urine samples. This will make it easier for us to understand the results of the urinary leukotriene measurement. If you like we will tell you the cotinine result at the end of the study. The amount of leukotriene in the urine may also vary with time or illness, so we will

² The study group will pay a £10 contribution to clinic visit travel expenses for you and your child.

collect a repeat sample if you come into hospital with wheeze, and also at the end of your year in the study at a time when your child is well. We will only do this if it is convenient to you.

Visit 2.

If you are happy to, and the doctor or nurse says you are suitable to take part, we will invite you to come for a second visit at a convenient time for you – normally within 2 weeks of the first visit. We may be able to visit your home, if you agree. We will then give you a box of sachets and instruction on how to use them. We will also give you a simple diary card to fill out when/if you have to use the medicine. The doctor or nurse will talk to you about it and answer any questions you have. There is space to write down anything you think is important for the nurse to know next time you see them. If you agree, we will let your GP know that your child has been enrolled into a study.

Your study doctor and/or nurse may ask your permission to make a sound recording of the interview when we give you the trial medicine and at the end of the study. This is because in a small number of parents we would like to find out their views on parent-guided medicines and whether we can improve the experience of parent and children in future studies. This is an “add on” study; you can take part in the main study without agreeing to this.

Phone Calls

You will be contacted once every two months by the research nurse. She will check whether you have used the trial medicine, and whether you have visited your GP or the Hospital. If your child has had an attack of wheeze she will ask you about the effect on the family, including things such as additional child care and days off work. If you have to use the trial medication we will ask you to post to us the completed diary card (and empty sachets) using a provided freepost envelope.

Replacement Medicines

If your child uses all their medicines, or the medicines reach their use-by date, we will contact you to provide you with a replacement box. If we do not contact you (or the medicines are lost or damaged) please contact us on the number provided. Do not attempt to get replacement medications from your GP or hospital doctor.

Extended Interview

If you have agreed to the optional extended interview a researcher will contact you and arrange a time that is convenient to you.

At 12 months

The study finishes for your child. We will ask you to send back all the used and unused medicine sachets.

What does my child have to do if we agree to take part?

- Your child will need to provide a saliva sample and urine sample(s).
- You should give your child the study medicine if they get a cold or wheeze attack
- There is nothing unpleasant or painful involved in the study.
- You will need to complete a symptom diary during attacks of wheeze.

You should tell the research doctor or nurse about any other medicines your child is taking. It is important to make sure that any other doctor your child visits knows that they are taking part in this study. Names and contact telephone numbers of the people running this study will be in the diary which is issued to you at your first visit. The study doctor will write to your GP and let them know that you are taking part in the research study.

What will happen when I start treatment?

- You will give your child 1 sachet of medication either directly into the mouth or mixed with cold or room temperature food from the start of every cold or wheezing attack.
- You will continue to give this every day for 10 days, even if your child gets better.
- You will complete a simple 10-day diary card for every course of medicine.
- If your child vomits after taking the medicine no additional dose should be given, and the vomit should be recorded on the diary chart.
- You should give all other medicines to your child as normal.
- You will inform the research team (see contact details) that you have commenced the study medicine by sending back the completed diary card at the end of the 10 days.

What are the alternatives for treatment?

Your child will receive the standard (normal) treatment for preschool wheeze of “as needed” inhaled salbutamol (the blue inhaler). If a doctor thinks that your child

needs to have regular inhaled steroids, these may be given without affecting the trial. If a doctor thinks that your child also needs daily montelukast, this can also be given, but in this case we will stop the trial medicine and, with your permission continue to collect information about the number of wheeze episodes.

What are the side effects of any treatment received when taking part?

- There are very few side effects reported with continuous montelukast. A possible side effect is a mild tummy upset and increased thirst. Some older children have had mild headaches.
- If your child accidentally takes too much montelukast the symptoms are similar to those already described. There may also be some increased sleepiness or agitation in some children. If your child takes an overdose of any medication you should seek medical advice. There is no evidence of longterm harm from montelukast overdose.

What are the other possible disadvantages and risks of taking part?

Some parents might worry that if their child is given the placebo (inactive) medicine they won't be getting enough medicine to manage their wheeze. However, every child in the study will get the normal standard care of inhaled salbutamol as well as other medicines that their doctor prescribes. Only children enrolled in the study are eligible to have "as required" montelukast.

What are the possible benefits of taking part?

During the study we will check that all of the children are well at every visit/phonecall. At any time during the study your GP or hospital doctor may decide to change your child's medicine or stop the study medicine. We are conducting this research so that we can know how best to treat children with preschool wheeze. We cannot promise that taking part will help your child personally, but your child will not be disadvantaged in any way. The information we get might help to improve the treatment of other children with preschool wheeze in the future.

What happens when the research study stops?

It may be some time after your child has completed the study before the results from all of the children taking part are known. We have to wait until the end of the whole study before we can analyse the results. Once the results are known we will write to you personally and tell your GP.

What if there is a problem?

Any complaint about the way you or your child have been dealt with during the study or any possible harm you might suffer will be addressed appropriately. Information relating to this is detailed in Part Two. If you have any complaints about this research study, please contact the appropriate Patient Advice and Liaison Service (PALS) office.

Will my child's taking part be kept confidential?

Yes. All of the information about your child's participation in this study will be kept confidential. The details are included in Part Two.

Contact details:

You will be able to contact a member of the research team to discuss any questions or concerns you may have and/or to get help.

Please call:

Research Nurse:

***** ***/*** *****

Tel:

*****/*****

Email:

*****/*****

Research Doctor:

***** ***/** *****

Tel:

Email:

Patient Advice and Liaison Service:

***** ***** ***** *****

*** ***** ***** *****

***** *****

***** ** **

Fax:

*** **** **

Minicom:

*** **** **

E-mail:

This completes Part One of the Information sheet. If the information in part One has interested you and you are considering participation, please continue to read the additional information in Part Two before making any decisions

Part Two

What if relevant new information becomes available?

Sometimes during the course of a research project, new information becomes available about the treatment/s being studied. If this happens, your study doctor will tell you and your child about it and discuss whether you both want to, or should, continue in the study. If you or your doctor decides that you should not carry on, your research doctor will discuss the reasons with you and make arrangements for your child's medical care to continue outside the study. If you decide to continue in the study you (and your child if appropriate) will be asked to sign updated consent forms. If the study is stopped for any other reason, you will be told why and your child's continuing care will be arranged.

What will happen if my child or I don't want to carry on with the research?

If at any point you decide to withdraw from the study, we will ask that you return all of the unused study medications to us. You can withdraw from treatment but continue to be followed up and have information collected.

Following withdrawal from the study, the research doctor will talk to you about whether they need to find out what medications your child was taking during the study to enable appropriate follow-on treatment. Your child will then be treated as per standard local clinical procedures. All data collected up until the time of withdrawal will be anonymised³ and included in the study analysis, unless you specifically state otherwise.

What if there is a problem?

- If you have a concern about any aspect of this study you should contact the researchers who will do their best to answer any questions (contact numbers are in Part One).
- If you are still unhappy after you have spoken to them and wish to complain formally, you can do this through the NHS Complaints Procedure.
- If you have a complaint about a study doctor or nurse you have seen at the hospital, you can contact the Patient Advice and Liaison Service (PALS) department at the hospital for help.
- If you wish to complain about a General Practitioner you have seen as part of this study, then you should contact the Primary Care Trust they belong to. Your study nurse will be able to help you with this if you want.

³ Anonymised means that a number will be used instead of your child's name so that no one will know that the information is about them.

- In the event that something goes wrong and your child is harmed during the research study the normal NHS complaints mechanism will be available to you. Additionally, if harm arises as a result of the design or management of this study, even if no one is at fault, you may have grounds for legal action against, or compensation from, the study sponsor: Queen Mary University of London. Please ask your doctor or research nurse for more information on this if you have any questions.
- If your child is harmed due to hospital staff negligence then you may have grounds for a legal action against the hospital where those staff are employed. However, you may have to pay your own legal costs.

Will my child's participation in this study be kept confidential?

- All information that is collected about you and your child during this study is considered to be confidential and giving this information to someone else ('a third party') is not allowed with the exceptions noted below.
- The paper files used to record information in this study will be labelled with a unique study number.
- Medical information may be given to your child's doctor or appropriate medical personnel responsible for their welfare.
- The paper files used to record information in this study will also be stored securely in a locked cabinet and the information will then be entered into a secure computer database file. This file will be labelled with your child's number but NOT their name. A copy of the information in the paper files will be stored securely by the research team at the coordinating study centre at Queen Mary University London. This is to ensure that all the information regarding the study remains accessible and secure for later analysis of the study results, and to check accuracy of information.
- When your child finishes taking part in the study we will need to find out what treatment they were taking so that they can inform your GP. To do this, we will have to link your child's trial number to their name but this link will still be kept separate to all of the other information collected about them in the study. The trial team will ensure that confidentiality is preserved.
- If you join the study, some parts of your child's medical records and the data collected for the study will be looked at by representatives of regulatory authorities and by authorised people from other NHS bodies to check that the study is being carried out correctly. Your child's medical records will be checked at the hospital and will not be removed. All authorised individuals have a duty of confidentiality to you and your child as research participants and nothing that could reveal your child's identity will be disclosed outside the research site. By signing the consent form you are giving permission for this to happen.
- In the event of the results of the study being sent to Health Authorities or published, all of your child's records will be kept confidential and your child's name will not be disclosed to anyone outside of the hospital.
- All documents and files relating to the study will be stored confidentially either at your local study site or the main study site or both for a maximum period of 20 years.

Involvement of the General Practitioner/family doctor (GP)

- With your consent, the study doctor will write to your child's GP to let them know that they are taking part in the study. In some circumstances your GP will already know since he/she will have sent out your invitation letter. The study doctor may ask your child's GP for further medical information about them if necessary.
- All patients (children) who are registered in the study will have follow up data collected about them. The information requested will all be related to your child's wheezing control and the research team will ask your GP to give them access to this data. By signing the attached consent form, you are agreeing for your GP to share this information with the research team.

I have private health insurance – does this make a difference?

You should inform your health insurance provider that your child has been enrolled into the study. They may wish to speak with the study group, in which case they can be provided with our contact details. Study involvement should not affect your insurance cover.

What will happen to any samples my child gives?

Your child's DNA and urine sample will be transferred to Queen Mary University London for testing and will be identified only by a special number to maintain your child's anonymity.

Will any genetic tests be done?

We will measure only the genes that affect how leukotrienes work in the body. Your child's sample will be collected by a researcher and sent directly to the laboratory at Queen Mary University London where it will be stored. Within 2 weeks we will measure the ALOX5 gene (a leukotriene gene). A DNA sample will be securely stored with a label that gives a subject number only (so that it cannot be directly linked to your child) and within 2 years sent to an external laboratory (KB Bio Science) for analysis of all the other genes that are associated with leukotrienes. Your child's sample will always be labelled with a special number, instead of their name, so no-one will know that it belongs to them. Once we have analysed it for leukotriene genes, the DNA sample will be disposed of and not kept.

What will happen to the results of the research study?

The results are likely to be published in the year following the end of the study. Your child's confidentiality will be ensured at all times and they will not be identified in any publication. At the end of the study the group results will be made available to you and/or your GP (should you wish). They will also be published on the National Institute of Health Research (NIHR) website.

Who is organising and funding the research?

The study is sponsored by Queen Mary University of London. This study is funded by the Efficacy and Mechanism Evaluation Programme of the Department of Health. Each participating research site has been allocated funds to pay for a researcher for this study, for the provision of general office supplies and to support pharmacy costs.

Who has reviewed the study?

The trial protocol has received the favourable opinion of the South East Research Ethics Committee

THANK YOU FOR READING THIS INFORMATION SHEET. WE HOPE YOU HAVE FOUND THE INFORMATION HELPFUL.

Appendix 2 Informed consent form

Serial Number: I _ I _ I - I _ I _ I _ I _ I

Parent/Guardian Consent Form (v5, 31.07.2012)

Please
initial box

- | | | |
|---|---|---|
| 1 | I confirm that I have read and understand the information sheet dated 31.07.12 (v5) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. | □ |
| 2 | I understand that my child's participation is voluntary and that I am free to withdraw at any time, without giving a reason, and without my care/my child's care or legal rights being affected. | □ |
| 3 | I understand that relevant sections of my child's medical notes and data collected during the study may be looked at by responsible individuals from the Barts and the London Clinical Trials Unit, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records. | □ |
| 4 | I agree to my child's GP being informed of my child's participation in the study. | □ |
| 5 | I agree to participate in a recorded interview about my views. ¹ | □ |
| 6 | I agree for my child to take part in this study. | □ |
-
- | | | |
|---|--|---|
| 7 | I do not wish my child to/my child is ineligible to take part in this study but I am happy for their information as recorded today to be used by the study team under the terms stated in the Information sheet. | □ |
|---|--|---|

¹ Delete if not applicable to this centre

Name of Patient		
_____	_____	_____
Name of Parent	Signature	Date
_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Principal Investigator	P.I. Countersignature	Date

1 copy for parent, 1 for researcher site file, 1 to be kept in patient (child's) notes

Appendix 3 Case report forms

All CRF copyright is owned by the WAIT trial team and the forms may be reproduced with appropriate accreditation and citation of their origin.

Two weeks prior to first investigational medicinal product dispensing assessment and randomisation case report form



T -2 ASSESSMENT & RANDOMISATION CRF		(Copy 1 – Trial Manager, Copy 2 – Local Site File)	
Serial number:	_ _ _ _ _	Site:	_ _ _ (e.g LO, AB, LE)
Researcher Initials:	_ _	Date of THIS Visit:	_ _ /_ _ /_ _
CONSENT TO USE DATA:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	IF NO – DO NOT COMPLETE
INCLUSION CRITERIA			
Age between 10 months and 5 years:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
Doctor-diagnosed wheeze, EVER:	Yes <input type="checkbox"/>	No <input checked="" type="checkbox"/>	
Wheeze in the preceding three months:	Yes <input type="checkbox"/>	No <input checked="" type="checkbox"/>	
At least two episodes of wheeze, EVER:	Yes <input type="checkbox"/>	No <input checked="" type="checkbox"/>	
Parent contactable by phone:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
EXCLUSION CRITERIA			
Regular Montelukast	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
History of neonatal chronic lung disease	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
In a drug trial in the preceding three months	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
Clinician-diagnosed chronic respiratory illness including structural airway anomaly and CF:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
Any other chronic illness predisposing to respiratory infection (including developmental delay with feeding difficulty):	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
If you have ticked any GREYED-OUT boxes do not register this child for the WAIT study			
INFORMED CONSENT TO ENTER STUDY:			
Parent and child information sheets reviewed:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	
Informed consent form signed:	Yes <input type="checkbox"/>	No* <input type="checkbox"/>	
*If no, please state the reason:			
Did not want to take part in a genetic study:	<input type="checkbox"/>		
Concerned about confidentiality:	<input type="checkbox"/>		
Other (please specify):	<input type="checkbox"/>		_____
If informed consent is <u>NOT</u> given do not collect samples, but please collect demographic data on page 2. If informed consent <u>IS</u> given collect samples as per guidance and also complete administration section on page 3			
STUDY VISIT CONDUCTED BY:			
Researcher Signature:	_____	Print Name:	_____ _ _ /_ _ /_ _
I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.			
PI Signature	_____	Print Name:	_____ _ _ /_ _ /_ _

WAIT T-2weeks, Assessment and Randomisation CRF v 6.0, 17/08/11

Data Entry Use Only: Date Received (DD/MM/YY): _/~/_ Entered (DD/MM/YY): _/~/_ Initials: _____

Monitoring Use Only: Database Cross-checked (DD/MM/YY): _/~/_ Initials: _____



T -2 ASSESSMENT & RANDOMISATION CRF		(Copy 1 – Trial Manager, Copy 2 – Local Site File)	
Serial number: _ _ _ _ Site: _ _ _ (e.g LO, AB, LE)			
Researcher Initials: _ _ Date of THIS Visit: _ _ / _ _ / _ _			
Weight: _ _ . _ _ kg	Height: _ _ _ . _ _ cm	DOB: _ _ / _ _ / _ _	Sex M <input type="checkbox"/> F <input type="checkbox"/>
Risk factors			
Birth, Atopy and Family History		Yes	No
Preterm Birth < 37wk gestation		<input type="checkbox"/>	<input type="checkbox"/>
Birth weight < 2500g		<input type="checkbox"/>	<input type="checkbox"/>
Allergy:			
Food		<input type="checkbox"/>	<input type="checkbox"/>
Drug		<input type="checkbox"/>	<input type="checkbox"/>
Itchy rash for > 6 months, ever		<input type="checkbox"/>	<input type="checkbox"/>
Eczema, ever		<input type="checkbox"/>	<input type="checkbox"/>
Tobacco Exposure:			
In utero		<input type="checkbox"/>	<input type="checkbox"/>
In household* (*any household smoking contact)		<input type="checkbox"/>	<input type="checkbox"/>
Daycare attendance		<input type="checkbox"/>	<input type="checkbox"/>
Immunisation Status:			
Pneumococcus		<input type="checkbox"/>	<input type="checkbox"/>
Influenza		<input type="checkbox"/>	<input type="checkbox"/>
History of Asthma			
Mother:		<input type="checkbox"/>	<input type="checkbox"/>
Father:		<input type="checkbox"/>	<input type="checkbox"/>
Pre-study Illness and Therapy			
Age at 1 st wheeze episode		_ _ y	_ _ m
		Yes	No
Wheezes only with viral URTI (episodic)		<input type="checkbox"/>	<input type="checkbox"/>
Wheezes at other times (multitrigger)		<input type="checkbox"/>	<input type="checkbox"/>
Interval between onset of URTI and wheezing:		_ _	hr
Admitted to hospital for wheeze:			
In last year?		<input type="checkbox"/>	<input type="checkbox"/>
Ever?		<input type="checkbox"/>	<input type="checkbox"/>
No of courses of systemic steroids in last year		_ _	
No of unscheduled medical attendances for wheeze in last year?		_ _	
Preventer therapy:			
None		<input type="checkbox"/>	<input type="checkbox"/>
Antileukotriene agents		<input type="checkbox"/>	<input type="checkbox"/>
Maintenance Inhaled Steroids		<input type="checkbox"/>	<input type="checkbox"/>
Episodic inhaled Steroids		<input type="checkbox"/>	<input type="checkbox"/>
Ethnicity			
Asian or Asian British <input type="checkbox"/> Bangladeshi <input type="checkbox"/> Indian <input type="checkbox"/> Pakistani <input type="checkbox"/> Any other Asian background	Mixed <input type="checkbox"/> White & Asian <input type="checkbox"/> White & Black African <input type="checkbox"/> White & Black Caribbean <input type="checkbox"/> Mixed other	Black or Black British <input type="checkbox"/> African <input type="checkbox"/> Caribbean <input type="checkbox"/> Any other Black background	White <input type="checkbox"/> British <input type="checkbox"/> Irish <input type="checkbox"/> White other
		Other Ethnic Group <input type="checkbox"/> Chinese <input type="checkbox"/> Any other ethnic group <input type="checkbox"/> I do not wish to disclose my ethnic origin	
Saliva sample collected:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Date collected: _ _ / _ _ / _ _
Saliva sample posted to laboratory:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Date sent: _ _ / _ _ / _ _
Urine sample collected:	Yes <input type="checkbox"/>	No <input type="checkbox"/>	Date collected: _ _ / _ _ / _ _
STUDY VISIT CONDUCTED BY:			
Researcher Signature: _____ Print Name: _____ _ _ / _ _ / _ _			
I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.			
PI Signature _____ Print Name: _____ _ _ / _ _ / _ _			

WAIT T-2weeks, Assessment and Randomisation CRF v 6.0, 17/08/11
 Data Entry Use Only: Date Received (DD/MM/YY): |_|_|/|_|_|/|_|_| Entered (DD/MM/YY): |_|_|/|_|_|/|_|_| Initials: _____
 Monitoring Use Only: Database Cross-checked (DD/MM/YY): |_|_|/|_|_|/|_|_| Initials: _____

T-2 VISIT RESEARCHER AIDE-MEMOIRE

BEFORE THE VISIT

- Ensure that you have access to:
 - a stadiometer and scales
 - universal containers and urine collection apparatus
 - Ice box and ice
 - a genotek kit
 - specimen label sheets
 - T-2 proforma
 - Consent form
 - Information sheets
- Ensure that appropriate language arrangements are in place if English is not the parents' first language.

DURING THE VISIT

CRF and consent

- Check that parents understand what you are saying, review information sheet and seek informed consent
- If informed consent is not granted then seek consent to use data short of administrative section
- Complete CRF up to the administrative section FOR ALL children (including weight and height), even if they do not agree to take part if consent is provided.
- Leave one copy of consent form with parents.
- Sign and gain PI countersignature on each page that is completed

Specimens – if informed consent gained

- Review sample collection guide
- Collect urine, decant into 2 x 1ml aliquots label with serial number and put on ice immediately.
- Finally collect and label DNA sample with serial number

Administration – if informed consent gained

- Complete the administration section on page 3 of the CRF including:
 - Administrative data
 - Checking and correcting Inhaler technique as necessary
 - Arranging T0 medicines dispensing visit
 - Signing off on CRF
 - Copy consent form and give a copy to parents

AFTER THE VISIT

CRF and consent

- Researcher completing to ensure their sign off is complete (*N.B.* researcher signing form must be delegated on the site delegation log to take consent/complete CRFs).
- CRF pages 1-2 to be countersigned by local PI, scanned and secure emailed via [redacted] to [redacted] in London as soon as possible (any delay will delay stratum allocation).
- Remember to keep one copy of consent form for local site file (consent and CRF) and give one copy to parents (consent form only).
- London Lab will allocate stratum to complete CRF T-2.

Specimens

- DNA sample to be posted urgently with request form in the pre-addressed envelope provided. An electronic copy of the request form must be sent to the trial coordinator on [redacted]
- Urine sample to be taken urgently on ice to be taken to local freezer and frozen at -70 or below for batch courier to London lab.

Stratification and Randomisation

- Trial laboratory technician should analyse DNA samples and complete stratification and inform researcher.
- PI should complete prescription with stratum based on above.
- Research nurse should deliver prescription to local pharmacy
- Local Pharmacist to complete prescription form, allocate IMP number and dispense trial drug for collection by local researcher
- Local researcher should convey IMP to parent.

WAIT T-2weeks, Assessment and Randomisation CRF v 6.0, 17/08/11

Data Entry Use Only: Date Received (DD/MM/YY): __/__/__ Entered (DD/MM/YY): __/__/__ Initials: _____

Monitoring Use Only: Database Cross-checked (DD/MM/YY): __/__/__ Initials: _____

T0 trial entry case report form



T0 TRIAL ENTRY CRF – PART A		(Copy 1 – Trial Manager, Copy 2 – Local Site File)
Serial number: _ _ _ _	Site: _ _ (e.g. AB, LE, LO etc.)	Subject Number (IMP): _ _ _ _
Patient Initials: _ _ _	Researcher Initials: _ _	Date of THIS Visit: _ _ / _ _ / _ _
1) CRF Documentation		
Tick if you have seen the signed and countersigned:		
<input type="checkbox"/>	i) Consent Form	
<input type="checkbox"/>	ii) CRF T-2	
<input type="checkbox"/>	iii) Prescription Form	
2) Samples		
Tick if you have:		
<input type="checkbox"/>	i) Collected DNA sample	
<input type="checkbox"/>	ii) Collected urine sample	
<input type="checkbox"/>	iii) Explained the need for additional urine samples on attendance at ED	
3) Diary Card		
Tick if you have:		
<input type="checkbox"/>	i) Provided and labelled diary cards (x5)	
<input type="checkbox"/>	ii) Explained their usage	
<input type="checkbox"/>	iii) Explained procedure for return	
4) Medication		
Tick if you or someone else have (on this or a prior visit)		
<input type="checkbox"/>	i) Checked salbutamol MDI and spacer availability	
<input type="checkbox"/>	ii) Checked MDI/spacer technique	
<input type="checkbox"/>	iii) Checked understanding of appropriate salbutamol usage	
<input type="checkbox"/>	iv) Checked IMP number matches number written by pharmacy on prescription	
<input type="checkbox"/>	v) Provided and explained use of IMP	
<input type="checkbox"/>	vi) Explained procedure for return of IMP	
<input type="checkbox"/>	vii) Explained procedure for replacement of IMP	
5) Communication		
Tick if you have:		
<input type="checkbox"/>	i) Provided local contact number and email	
<input type="checkbox"/>	ii) Explained indications for contact (solely trial-related and including suspected drug reactions , contact local NHS for acute health advice).	
<input type="checkbox"/>	iii) Provided pre-addressed jiffy bag for return of IMP/empty salbutamol canisters/diary cards	
Researcher Signature: _____		Date: _ _ / _ _ / _ _
Print Name: _____		
I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.		
PI Signature: _____		Date: _ _ / _ _ / _ _
Print Name: _____		

WAIT T0 checklist and CRF v3.0, 23/6/11

CTU Use Only: Date Received (DD/MM/YY): |_|_|/|_|_| Entered (DD/MM/YY): |_|_|/|_|_| Initials: _____

T0 researcher aide-memoire



T0 VISIT RESEARCHER AIDE-MEMOIRE

<p>BEFORE THE VISIT</p> <ul style="list-style-type: none"> • Ensure that you have seen the signed and countersigned consent form, CRF T-2, and prescription form • Ensure that you have collected the trial drug in good time from your local pharmacy and that the subject number on the box matches that on the prescription and CRF T0. • Ensure that you have a copy of the CRF T0 and have completed sections 1 and 2 in advance. • Ensure that you have copies of the diary card and understand its use. • Ensure that appropriate language arrangements are in place if English is not the parents' first language.
<p>DURING THE VISIT</p> <p>Diary Card</p> <ul style="list-style-type: none"> • Explain the use of the diary card • Provide one diary card per course of medication (usually five per box) • Explain diary card return procedure <p>Medication</p> <ul style="list-style-type: none"> • Check the parents' possession of and knowledge of the use of spacer and MDI (may be brief if already performed e.g. on ward/at T-2 visit) • Give IMP to parents and explain when and how to use and return it <p>Communication</p> <ul style="list-style-type: none"> • Give advice and information regarding researcher contact (including email and phone contact numbers – in PIS). • Explain what to do if there are concerns regarding drug reactions or trial participation (contact local healthcare provider if child acutely unwell, contact researcher otherwise). <p>T0 Trial Entry CRF</p> <ul style="list-style-type: none"> • Work systematically through CRF
<p>AFTER THE VISIT</p> <ul style="list-style-type: none"> • Researcher completing to ensure their sign off is complete (<i>N.B.</i> researcher signing form must be delegated on the site delegation log to take consent/complete CRFs) • Keep one copy of the CRF in the local site file, send one copy to the London Trial coordinator. • Send a recruited/not recruited letter to the GP and put a copy of this and the consent form in the clinical notes if appropriate.

WAIT T0 checklist and CRF v3.0, 23/6/11

CTU Use Only: Date Received (DD/MM/YY): __/__/__ Entered (DD/MM/YY): __/__/__ Initials: _____

2 months to 12 months prior to time of first investigational medicinal product dispensing telephone call case report form

NHS
National Institute for
Health Research

WAIT

Barts and The London
Queen Mary's School of Medicine and Dentistry
UNIVERSITY OF ABERDEEN **University of Leicester**

BIMONTHLY PHONECALL CRF		(Copy 1 – Local Site file – <u>send to trial manager on completion</u>)	
Serial number:		Site:	
Patient Initials:		Subject Number(IMP):	
Phonecall T+2m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
Phonecall T+4m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
Phonecall T+6m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
		(returns reminder) <input type="checkbox"/>	
Phonecall T+8m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
Phonecall T+10m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
Phonecall T+12m	Date / /	Time : (24hr)	Caller Initials
Number of IMP initiations?	Total days used?	Number and dates (below) of GP/Hosp attendances	
Indications reminder <input type="checkbox"/> Diary card reminder <input type="checkbox"/>	Number of days school/childcare missed	Hosp name / , / , / , /	
Days taken off work (any carer)	Other medications used _____	GP name / , / , /	
		Adverse Events: <input type="checkbox"/> (if ticked complete SAE proforma)	
		(returns reminder) <input type="checkbox"/> <input type="checkbox"/> <input type="checkbox"/>	
		IMP was helpful Y N	
Parent Signature: _____		Print Name: _____	
Date: / /			
Researcher Signature: _____		Print Name: _____	
Date: / /			
I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.			
PI Signature: _____		Print Name: _____	
Date: / /			

WAIT bimonthly phonecall proforma, v1, 180810

2 months to 12 months prior to time of first investigational medicinal product dispensing medical attendance verification case report form



MEDICAL ATTENDANCE VERIFICATION (Copy 1 – Local File – send to trial manager on completion)					
Serial number:		Site:			
Patient Initials:		Subject Number(IMP):			
Phonecall T+2m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1	H/G				
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Phonecall T+4m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Phonecall T+6m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Phonecall T+8m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Phonecall T+10m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Phonecall T+12m	Date / /	Time : (24hr)	Caller Initials		
	Hosp/GP Name	Date (of admission)	(Date of Discharge)	Verified?	Comments (+ indicate H or GP)
Attendance 1					
Attendance 2					
Attendance 3					
Attendance 4					
Attendance 5					
Attendance 6					
Parent Signature: _____	Print Name: _____	Date: / /			
Researcher Signature: _____	Print Name: _____	Date: / /			
I have reviewed all data in this CRF and verify that the contents are consistent with observations and source records.					
PI Signature: _____	Print Name: _____	Date: / /			

WAIT bimonthly phonecall proforma, v1, 180810

Diary card case report form


WAIT

 National Institute for Health Research

PARENT DIARY CARD

Patient Serial Number __ - ____

INSTRUCTIONS

START the trial medicine when your child has a **COLD** or you think they will have a **WHEEZE** attack

CONTINUE it for **10 days** even if your child is well

- **Complete this diary card every time you start the trial medicine**
- **Complete the card at the END of each day for 10 days**
- **Stop the card when you stop the trial medicine**
- **Post the card back to us in the freepost envelope**
- **Remember to send back your empty sachets with this card.**

Subject No. (IMP) _ _ _ _ _ _ _	Study Site _ _ _	Card Number _ _ _
Patient Initials: _ _ _ _	Researcher Initials: _ _ _	Date Given/Posted _ _ / _ _ / _ _

Parent Initials |_|_|_|

WAIT DIARY CARD, v4 100112

DAY 1 OF MEDICINE

SUN MON TUES WED THUR FRI SAT

DATE: I _ I _ / _ I _ / 20 _ I _ I TIME: _ I _ : _ I _

The questions below refer to the past 24 hours.

Please answer as well as you can remember

Please answer the questions by ticking (v) Yes or No →	Y	N
Did your child wheeze in the last 24 hours?		
Did your child have a cold in the last 24 hours?		
Did you give your child the TRIAL medicine TODAY?		
Did your child vomit the medicine TODAY?		
Did your child miss school or nursery TODAY?		
Did ANYONE stay home to look after your child TODAY?		
Did your child see a doctor or nurse TODAY?		
Did you give your child the blue inhaler in the last 24 hrs		
If yes? How many times did you give it to them in the last 24 hours?		
On average, how many puffs did you give them <u>each time</u> ?		

Parent Initials I _ I _ I

WAIT DIARY CARD, v4 100112


WAIT

 National Institute for Health Research

DAY 10 OF MEDICINE

SUN MON TUES WED THUR FRI SAT

DATE: I__I__/_I__/_20__I__I TIME: __I__:__I__

The questions below refer to the past 24 hours.

Please answer as well as you can remember

Please answer the questions by ticking (✓) Yes or No →	Y	N
Did your child wheeze in the last 24 hours?		
Did your child have a cold in the last 24 hours?		
Did you give your child the TRIAL medicine TODAY?		
Did your child vomit the medicine TODAY?		
Did your child miss school or nursery TODAY?		
Did ANYONE stay home to look after your child TODAY?		
Did your child see a doctor or nurse TODAY?		
Did you give your child the blue inhaler in the last 24 hrs		
If yes? How many times did you give it to them in the last 24 hours?		
On average, how many puffs did you give them <u>each time</u> ?		

Parent Initials I__I__I

WAIT DIARY CARD, v4 100112



TRIAL MEDICINE COMMENTS

(write anything you would like to tell us about the medicine)

OTHER MEDICINES TAKEN THIS WEEK

Medicine	Dose	Days	Doses per day

THANK YOU FOR COMPLETING THIS DIARY.

NOW PLEASE RETURN IT IN THE FREEPOST ENVELOPE PROVIDED. THIS WILL INFORM YOUR RESEARCHER THAT YOU HAVE USED THE MEDICINE.

RESEARCHER PHONE NUMBER _____

Parent Initials |__|__|

WAIT DIARY CARD, v4 100112

Non-serious adverse event case report form



NSAE - SINGULAIR							
Site number:		Subject number:		Researcher Initials:			
Patient Initials:		Date of THIS Visit/Call: / /					
NONSERIOUS ADVERSE EXPERIENCE							
 <p>If AE resulted in Death, if AE is immediately Life-Threatening, results in Persistent or Significant Disability/Incapacity, results in Hospitalization or Prolongs an Existing Hospitalization, is a Congenital Anomaly/Birth Defect, a Cancer, the result of an Overdose, or Other Important Medical Event, enter event on the SAE form.</p>							
Were there any nonserious AEs since last visit/phonecall? None <input type="checkbox"/> or complete the form below Date information obtained: <u>DD-Mon-YYYY</u>							
Clinical AE Term	Check if Worsening Condition	Onset Date	Stop Date (or check box if continuing)	Duration (if less than 24 hours)	Intensity	Action Taken on Primary Test Drug Due to AE:	Did primary test drug cause AE? (Refer to Guidelines for Causality)
		DD Mon-YYYY	DD Mon-YYYY	Specify number of hours minutes or seconds Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>	1 = Mild 2 = Moderate 3 = Severe	1 = None 2 = Interrupted 3 = Discontinued 4 = Reduced	1 = Definitely not 2 = Probably not 3 = Possibly 4 = Probably 5 = Definitely
	<input type="checkbox"/>		<input type="checkbox"/> Continuing	Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>			
	<input type="checkbox"/>		<input type="checkbox"/> Continuing	Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>			
	<input type="checkbox"/>		<input type="checkbox"/> Continuing	Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>			
	<input type="checkbox"/>		<input type="checkbox"/> Continuing	Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>			
	<input type="checkbox"/>		<input type="checkbox"/> Continuing	Hrs <input type="checkbox"/> Min <input type="checkbox"/> Sec <input type="checkbox"/>			
NONSERIOUS LABORATORY or OTHER DIAGNOSTIC PROCEDURES							
TYPE OF A E	LABORATORY OR OTHER DIAGNOSTIC PROCEDURES (To describe Lab AE use the term "Increased" or "Decreased")	Check if Worsening Condition	Date lab specimen obtained or special exam performed	Action Taken on Primary Test Drug Due to AE:	Did primary test drug cause AE? (Refer to Guidelines for Causality)		
			DD Mon-YYYY	1 = None 2 = Interrupted 3 = Discontinued 4 = Reduced	1 = Definitely not 2 = Probably not 3 = Possibly 4 = Probably 5 = Definitely		
Lab <input type="checkbox"/>		<input type="checkbox"/>					
Other <input type="checkbox"/>		<input type="checkbox"/>					
Lab <input type="checkbox"/>		<input type="checkbox"/>					
Other <input type="checkbox"/>		<input type="checkbox"/>					
Lab <input type="checkbox"/>		<input type="checkbox"/>					
Other <input type="checkbox"/>		<input type="checkbox"/>					
Comments:							

WAIT SAE Proforma, Version Number v1, 25/08/10

Serious adverse event case report form

SERIOUS ADVERSE EVENT REPORTING FORM

(BLT/QM sponsored trial)

Once you have become aware of a SAE or SUSAR, please scan & email/fax this signed form to the Research Governance & GCP Manager: [REDACTED] (or to the trial co-ordinator's fax number if multi site project) WITHIN a working day of learning of the event for SUSARs and within the time line outlined in the protocol approved by the MHRA and REC if expected SAEs. It is the CI's responsibility to inform the MREC of the SUSARs. If this event is a SUSAR, request an acknowledgment email of receipt of this form, from the JRO, print it and file it in your TMF.

Report type:	Initial	Follow up
If the project is multi-site, the section below should be completed by the Main site Trial coordinator prior to sending the template to the sites		
Full title of the study:	Parent-determined oral montelukast therapy for preschool wheeze with stratificatiobn for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.	
Name of sponsor:	BLT	QMUL
Sponsor R&D Number:	EudraCT Number:2009-015626-11	
MREC Number:	09/H1102/110	
Chief Investigator:	Name: Prof J Grigg	Phone No: [REDACTED]
	Email address: [REDACTED]	
Is this a double blind study?	Yes	
	If Yes are the code break procedures in place with pharmacy? Yes	
Name of ALL IMPs and/or medical devices	IMP 1: Montelukast	IMP 3: IMP 4:
	IMP 2:	
This section should be completed by the SITE:		
Subject identification code:	Patient/initials (first, last):	_____
DOB: (Day/Month/Year) (___ / ___ / ___)	Sex:	M F
Patient's Age:		
Principal Investigator:	Name:	Phone No:
	Email address:	
Trial Co-ordinator local site:	Name:	Phone No
	Email address:	
Name of reporting host institution:	Trust/ Institution name: Site number:	
Date of site becoming aware of the event	Onset date of SAE:	Resolution date of SAE:
Event Description (e.g. body site, symptoms) (*please use separate form for each event)	Event*:	Severity: Mild Moderate Severe
Type of SAE	Results in Death Life threatening Hospitalisation or prolongation of hospitalisation Persistent or significant disability or incapacity Congenital anomaly or birth defect "Other" important medical event If "Other", please describe:	
	The co-ordinator needs to replace IMP 1,2,3,4 ... by the actual name of the IMP prior to sending the template to the sites.	
Is the SAE likely to be a reaction to one of the	IMP 1 likely or possibly Related	Unrelated
	IMP 2 likely or possibly Related	Unrelated

SAE reporting form V4, 22/12/08

IMPs or medical device in the trial?	IMP 3 likely or possibly Related	Unrelated
	IMP 4 likely or possibly Related	Unrelated
Is the SAE expected? <i>Expected reactions will be found in the Investigator Brochure, SmPC(http://emc.medicines.org.uk/) and/or protocol.</i>	IMP 1 Expected	Unexpected
	IMP 2 Expected	Unexpected
	IMP 3 Expected	Unexpected
	IMP 4 Expected	Unexpected
Is the SAE due to the progression of an underlying illness?	Yes No	Is the SAE related to the trial CONDUCT? Yes <input type="checkbox"/> No <input type="checkbox"/>
Names of non IMPs concomitant medicines:		
Names of concomitant diseases:		
Is the event classified as a SUSAR? (ie, RELATED to one of the IMPs and UNEXPECTED)	Yes No	If Yes, please also complete CIOMS form http://www.jazmp.si/files/farmakovigilanca/ObrazecPoro%C4%8DanjeNUZ_CIOMS_angl.doc , also on page 4. If Yes, please give the batch number of each of the IMPs related to the SAE: IMP 1: Batch Number: IMP 2: Batch Number: IMP 3: Batch Number: IMP 4: Batch Number:
Action taken with study treatment:	IMP 1 Continued Reduced Increased Temporary stop Permanent stop*	
	IMP 2 Continued Reduced Increased Temporary stop Permanent stop*	
	IMP 3 Continued Reduced Increased Temporary stop Permanent stop*	
	IMP 4 Continued Reduced Increased Temporary stop Permanent stop*	
Did the PI withdraw the patient from the study?	Yes No	
Outcome of SAE:	Resolved Resolved with sequelae* *specify sequelae _____	
	Improved Persisting Worsened Fatal (date of death ___ / ___ / ___) Unknown	
	If fatal, copy of post-mortem available? Yes No	
Person completing the form if not the PI	Name: Phone No	
	Email address: Date:	
	Signature: Date:	
Investigator's Name:	Print :	
Investigator's Signature	Date:	

Additional information requested by the CI's team for this project:

	CI's team, please customise this table prior to sending the form to the sites.
	Please add as many rows as required.

For Multi-site trials only

Date form RECEIVED by CI's team from external site: (___ / ___ / ___) (This date will be DAY 1 for SUSARs)	Reviewed by:	Date:
---	--------------	-------

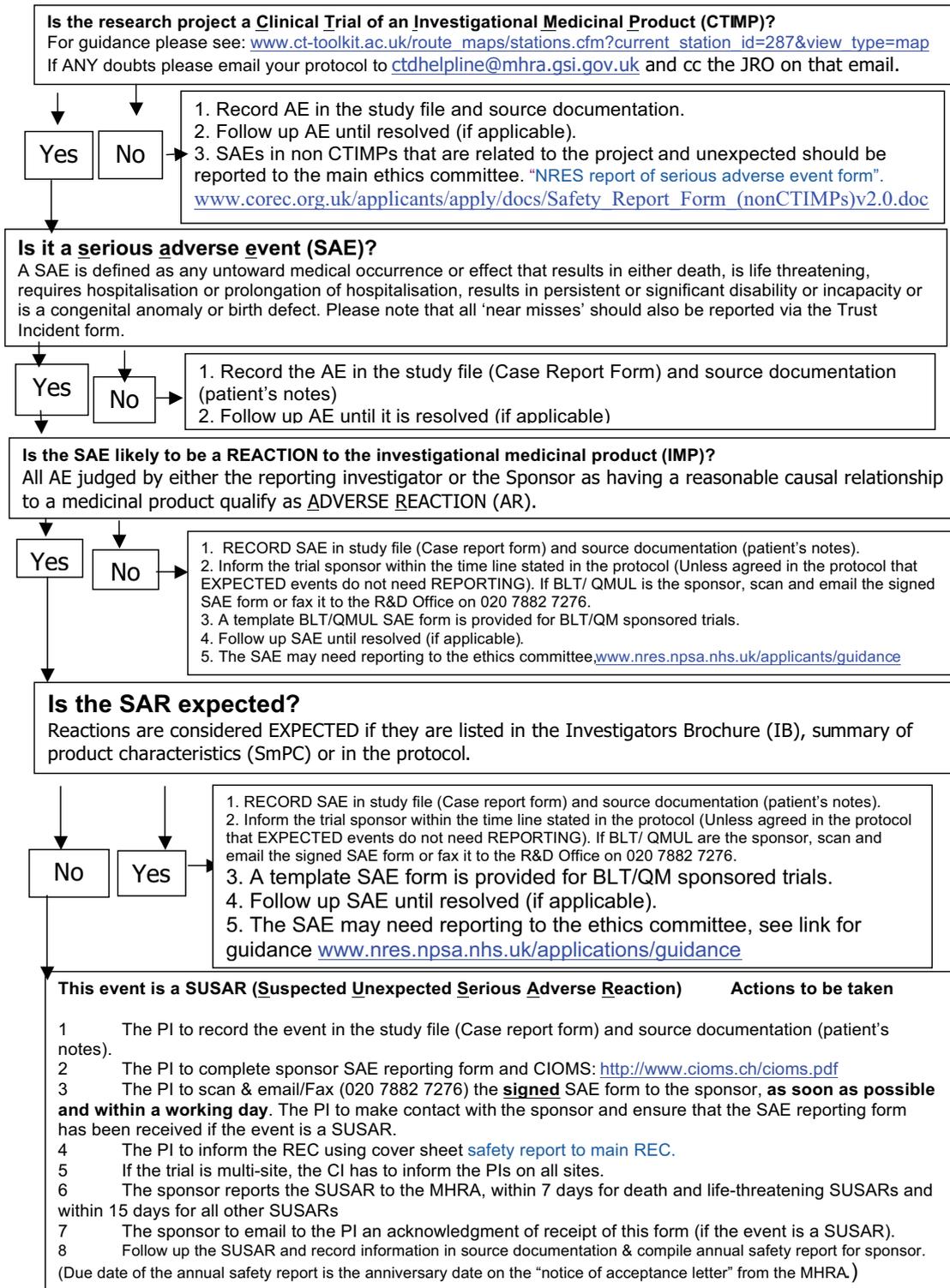
For R&D Office use only

Date form RECEIVED by R&D team: (___ / ___ / ___)	Reviewed by:	Date reviewed:
For SUSAR only:	Date reported to the MHRA:	

SAE reporting form V4, 22/12/08The CI cannot downgrade SUSARs reported by the treating PI at the site

Adverse Event (AE) Recording & Reporting

An AE occurs during a RESEARCH project, what do I do next?



SAE reporting form V4, 22/12/08 The CI cannot downgrade SUSARs reported by the treating PI at the site

CIOMS

http://www.jazmp.si/files/farmakovigilanca/ObrazecPoro%C4%8DanjeNUZ_CIOMS_angl.doc

SUSPECT ADVERSE REACTION REPORT										
I. REACTION INFORMATION										
1. PATIENT INITIALS	1a. COUNTRY	2. DATE OF BIRTH			2a. AGE	3. SEX	4-6 REACTION ONSET			8-12 CHECK ALL
(first, last)		Day	Month	Year	Years		Day	Month	Year	APPROPRIATE TO ADVERSE REACTION
7 + 13 DESCRIBE REACTION(S) (including relevant tests/lab data)										<input type="checkbox"/> PATIENT DIED <input type="checkbox"/> INVOLVED OR PROLONGED INPATIENT HOSPITALISATION <input type="checkbox"/> INVOLVED PERSISTENT OR SIGNIFICANT DISABILITY OR INCAPACITY <input type="checkbox"/> LIFE THREATENING <input type="checkbox"/> CONGENITAL ANOMALY <input type="checkbox"/> OTHER MEDICALLY IMPORTANT CONDITION
II. SUSPECT DRUG(S) INFORMATION										
14. SUSPECT DRUG(S) (include generic name)						20. DID REACTION ABATE AFTER STOPPING DRUG? <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> NA				
15. DAILY DOSE(S)				16. ROUTE(S) OF ADMINISTRATION		21. DID REACTION REAPPEAR AFTER REINTRO- DUCTION? <input type="checkbox"/> YES <input type="checkbox"/> NO <input type="checkbox"/> NA				
17. INDICATION(S) FOR USE										
18. THERAPY DATES (from/to)				19. THERAPY DURATION						
III. CONCOMITANT DRUG(S) AND HISTORY										
22. CONCOMITANT DRUG(S) AND DATES OF ADMINISTRATION (exclude those used to treat reaction)										
23. OTHER RELEVANT HISTORY (e.g. diagnoses, allergies, pregnancy with last menstrual period, etc.)										
IV. MANUFACTURER INFORMATION										
24a. NAME AND ADDRESS OF MANUFACTURER					26-26a. NAME AND ADDRESS OF REPORTER (INCLUDE ZIP CODE)					
ORIGINAL REPORT NO.		24b. MFR CONTROL NO.								
24c. DATE RECEIVED BY MANUFACTURER		24d. REPORT SOURCE <input type="checkbox"/> STUDY <input type="checkbox"/> LITERATURE <input type="checkbox"/> HEALTH PROFESSIONAL <input type="checkbox"/> REGULATORY AUTHORITY <input type="checkbox"/> OTHER								
DATE OF THIS REPORT		25a. REPORT TYPE <input type="checkbox"/> INITIAL <input type="checkbox"/> FOLLOW-UP								

SAE reporting form V4, 22/12/08The CI cannot downgrade SUSARs reported by the treating PI at the site

Serious Adverse Event (SAE) Reporting Form, Guidance notes:

1. Please see the SAE flowchart (page 3) for assistance.
2. The BLT/ QMUL SAE reporting form detailed on page 1 of this document needs to be completed if a SAE occurs during a BLT/QM sponsored clinical trial. If BLT/QM is not the sponsor please contact the sponsor and follow the sponsor's SOP.
3. SUSAR's should be reported to the sponsor immediately as the sponsor has a legal obligation to report this to the MHRA within 7 days (for fatal or life-threatening SUSAR's) or 15 days for all other SUSAR's. The PI needs to fill in the CIOMS form which will also be forwarded to the MHRA.

4. SAE REPORTING IN MULTI-SITE STUDIES

In multi-site studies, the PI at each external site should fax this form to the CI at the BLT/QMUL site. The CI and study team should check that ALL fields have been completed and that the form has been signed by the PI at that site. **The CI should not down grade SAEs or SUSARs from the treating PI at the site.** However the CI can upgrade an AE to a SAE or a SAE to a SUSAR. The CI should then scan, email or fax the completed form to the R&D office within a working day of becoming aware of the event.

SAE reporting form V4, 22/12/08The CI cannot downgrade SUSARs reported by the treating PI at the site

Withdrawal case report form



TRIAL WITHDRAWAL CRF		
Serial number: _ _ _ _	Site: _ _ (AB, LE, LO, GP)	Subject number (IMP): _ _ _ _
Patient Initials: _ _ _ _	Researcher Initials: _ _ _	Date of THIS Visit: _ _ / _ _ / _ _

(Circle as appropriate)

1.	Has the participant withdrawn from:	Treatment Only <i>(i.e. Placebo/Montelukast)</i>	0						
		Trial <i>(i.e. Treatment and Follow-Up)</i>	1						
2.	Date of withdrawal	<table border="1"> <tr> <td> _ </td> <td> _ </td> <td> _ _ _ </td> </tr> <tr> <td>Day</td> <td>Month</td> <td>Year</td> </tr> </table>	_	_	_ _ _	Day	Month	Year	
_	_	_ _ _							
Day	Month	Year							
3.	Reason for withdrawal <i>(Circle all that apply)</i>	Eligibility criterion no longer met (Specify: _____)	1						
		Death of participant (SAE no. _____)	2						
		Other adverse event (AE/SAE no. _____)	3						
		Deterioration of pre-existing medical condition	4						
		Poor adherence to treatment	5						
		Perceived lack of efficacy of medication	6						
		Unable to locate participant/carer	7						
		Other (Specify: _____)	8						
4.	Withdrawal decision initiated by: <i>(Circle all that apply)</i>	Chief Investigator (CI)	1						
		Principal Investigator (PI)	2						
		Referring Investigator	3						
		Carer	4						
		Participant	5						
		Other (Specify: _____)	6						
5.	Would the PI have independently recommended treatment withdrawal?	No	0						
		Yes	1						
6.	Permission given to use data collected:	No, use of all data collected to date denied	1						
		Yes, partial permission to use data up to withdrawal (Specify: _____)	2						
		Yes, permission to use all data up to withdrawal	3						
		Yes, permission to collect and use all follow-up data	4						
7.	Treatment code broken: <i>(Not unless absolutely necessary)</i>	No	0						
		Yes (Emergency Unblinding Request no. _____)	1						
8.	Signature of Researcher								
	Signature of Principal Investigator								

WAIT Study Withdrawal Form – version 4.0 – 25/08/11

Appendix 4 Laboratory procedures

Laboratory quality assurance standard operating procedure (London)



Standard Operating Procedures (SOP) for: WAIT Trial QA/QC			
SOP Number:	12	Version Number:	1
Effective Date:		Review Date:	

Author:	Iain Dickson
---------	---------------------

Authorisation:	
Name / Position	Dr Tom Vulliamy
Signature	
Date	

Purpose and Objective:
To document quality assurance/quality control (QA/QC) procedures taking place within the WAIT trial.

SOP Text

	Responsibility	Activity
1.	Lab Technician	Sample Receipt – When a sample arrives, it is checked over for packaging, labelling and for any leaks. This is documented for each sample in the ‘WAIT Sample Receipt’ log, kept in filing cabinet GWHD-6, in the paediatric write-up area.
2.	Lab Technician	Sample Processing – All samples are amplified in duplicate. All samples are also run alongside positive standards. Three of these standards were used to validate the method (see ‘Method Validation’ in the WAIT trial lab site file) and were sequenced to confirm their genotype. They are as follows: S1-W001 – 5/5 genotype S2-2535 – 4/5 genotype S3-2551 – 3/5 genotype A fourth standard with the 5/6 genotype is also run with all samples. This standard originated from a trial sample which was found to have the 5/6 genotype. DNA from this saliva sample was re-extracted and is labelled with the same trial number followed by a (2), e.g. LO-140(2). As a standard, it will therefore appear on the genotyping worksheet as, for example, S4-LO140(2).
3.	Lab Technician	Repeat Testing – When there is a low number of samples to be analysed and space on the genotyping plate, randomly picked old trial samples are re-amplified and re-genotyped. This is demonstrated on the genotyping worksheet by ‘QA/QC’ in the margins next to the samples being re-run. Periodically, a whole ‘QA/QC’ run may take place where all the samples on the plate are re-genotyped. This again will be denoted by ‘QA/QC’ on the worksheet.
4.	Lab Technician	Results Reporting – All results in the WAIT trial are double checked by another member of the lab staff, Dr. Tom Vulliamy. Before a report or sample result is sent out, Dr. Vulliamy will look over the raw data and double check the genotype result, as well as the stratification. Please see the ‘Results Reporting SOP’.

Urinary Eicosanoid – high-performance liquid chromatography–tandem mass spectrometry standard operating procedure (Krakow)

Marek Sanak and Anna Gielicz 2 August 2009

Standard operating procedure: urinary eicosanoids measurements

Platform

- High-performance liquid chromatography–mass spectrometry.
- Gas chromatography–mass spectrometry.

Sample requirements:

- Frozen urine, two aliquots of 1 ml (Eppendorf tubes).

Sample preparation

Urine (from the first morning micturition or sampled using a schedule of the clinical protocol) immediately transferred to the laboratory in a 50-ml disposable jar.

Preprocessing

If clear, aliquot into two 1-ml Eppendorf tubes and label. Store frozen at -70°C . If not clear, centrifuge at 5000 g for 10 minutes in a swinging bucket rotor, then aliquot.

Stability

Tested for 2 years' storage; no decay of eicosanoids.

Urinary creatinine measurement

Use one aliquot, thaw on ice or in the fridge (4°C , 3–5 hours). Measure 200 μl using the standard protocol and Vitros 350 Chemistry System (Ortho Clinical Diagnostics, High Wycombe, UK).

Organic phase extraction

Thaw on ice or in the fridge (4°C , 3–5 hours; batch up to 20 samples). Adjust pH to 3.5 with 1 N HCl (30–80 μl), check pH using the narrow-range pH stick. Add internal deuterated standards mix containing: $\text{LTE}_4\text{-d}_3$ (2 ng), tetranor-PGE-M- d_6 (10 ng), tetranor-PGD-M- d_6 (10 ng), 13,14-dehydro,15-keto PGE $_2$ - d_4 (1 ng), 13,14-dehydro,15-keto PGD $_2$ - d_4 (1 ng), 13,14-dehydro,15-keto-tetranor-PGE $_2$ - d_4 (1 ng), 13,14-dehydro,15-keto-tetranor-PGD $_2$ - d_4 (1 ng), 9α 11 β -PGF $_2$ - d_4 (1 ng), 15-deoxy, δ -12,14-PGJ $_2$ - d_4 (1 ng) in methanol – 10 μl of the mix. If uric acid precipitate is present, spin for 10 minutes at 10,000 g at 4°C (in a microcentrifuge) and transfer the supernatant to a fresh tube. Mix in a conical 10-ml tube with 1 ml of tertiary-butylmethyl-ether (TBM), vortex for 2 minutes, spin as before. Collect upper organic phase to fresh tube, repeat extraction with another 1 ml of TBM, combine organic phases. Dry at room temperature under nitrogen flow (1 l/minute) for 30 minutes. Dissolve in 60 ml of methanol and immediately proceed with analysis.

High-performance liquid chromatography–tandem mass spectrometry equipment

Autosampler (Shimadzu Siil-2-AC; Shimadzu Europa GmbH, Duisburg, Germany), reverse phase column (Zorbax Eclipse XDB C-18; Agilent Technologies, Santa Clara, CA, USA) stabilised thermally at 37°C , multiple reaction monitoring mode (MRM) tandem mass spectrometry (Qtrap 4000; Applied Biosystems, MA, USA) equipped with electrospray ion source negative ionisation mode, use batch protocol for urinary eicosanoids.

Test: inject 10 μl of internal standard mix. Check for area under the peak $> 20,000$.

Injection

10 µl of methanol extract

Elution

Gradient consisting of two mobile phases: (1) acetonitril/water/acetic acid (20/80/0.01) and (2) acetonitril/isopropanol/acetic acid (55/45/0.01) using the flow rate of 0.11 ml/minute.

Gas chromatography–mass spectrometry

Equipment

Single quadrupole mass spectrometer (Engine 5989B series II; Helwett Packard, Palo Alto, CA, USA), 15-m capillary column, gas-chromatography negative-ion chemical ionisation mode (GC-NICI-MS). Use protocol for urinary prostanoids.

Three step derivatisation

To pentafluorobenzyl ester, trimethylsilyl esters, and methoxyoxime, and subsequent purification by thin-layer chromatography (TLC).

Following methanol elution from the silica of TLC.

2 µl of the eluate.

Data analysis

High-performance liquid chromatography–mass spectrometry

Ion pairs

- $\text{LTE}_4\text{-d}_3$ 441–336 and LTE_4 438–333
- tetranor-PGE-M- d_6 tetranor-PGD-M- d_6 333–315 and tetranor-PGE-M tetranor-PGD-M 327–309 (different retention time)
- 13,14-dehydro,15-keto $\text{PGE}_2\text{-d}_4$ and 13,14-dehydro,15-keto $\text{PGD}_2\text{-d}_4$ 355–337 and 13,14-dehydro,15-keto PGE_2 and 13,14-dehydro,15-keto PGD_2 351–333 (different retention time)
- 13,14-dehydro,15-keto-tetranor- $\text{PGE}_2\text{-d}_4$ and 13,14-dehydro,15-keto-tetranor- PGD_2 301–283 and 13,14-dehydro,15-keto-tetranor- PGE_2 and 13,14-dehydro,15-keto-tetranor- PGD_2 297–279 (different retention time)
- 15-deoxy,delta-12,14- $\text{PGJ}_2\text{-d}_4$ 319–275 and 15-deoxy,delta-12,14- PGJ_2 315–271.

Gas chromatography–mass spectrometry

- $9\alpha 11\beta\text{-PGF}_2\text{-d}_4$ 573 and $9\alpha 11\beta\text{-PGF}_2$ 569.

Integrate area under the peak (AUP) for the analyte and corresponding internal standard (IS). Calculate from the formula: $\text{IS}_{\text{amount}} \times (\text{AUP}_{\text{analyte}}/\text{AUP}_{\text{IS}})$. Report as divided by urinary creatinine concentration in pg/mg creatinine.

Appendix 5 Statistical analysis plan

The statistical analysis plan was finalised prior to locking of the trial database.

Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype (WAIT) **Statistical Analysis Plan**

Version: 2.0

Date: 18th February 2014

Person(s) contributing to the analysis plan	
Name(s) and position(s)	Clare Rutterford Trial Statistician Sandra Eldridge Senior Statistician Chinedu Nwokoro
Authorisation	
Position	Chief or principal investigator
Name	Jonathan Grigg
Signature	
Date	
Position	Senior trial statistician
Name	Sandra Eldridge
Signature	
Date	
Position	Independent statistician*
Name	NA
Tick once reviewed	NA
Date	DD/MMM/YYYY

*This will normally be the Trial Steering Committee (TSC) statistician, but if there is no TSC the DMC statistician may sign off the analysis plan, provided there has been no interim unblinded analysis.

INTRODUCTION

Purpose of statistical analysis plan

The purpose of this document is to provide details of the statistical analyses and presentation of results to be reported within the principal paper(s) of the WAIT trial. Subsequent papers of a more exploratory nature (including those involving baseline data only) will not be bound by this strategy but will be expected to follow the broad principles laid down in it. Any exploratory, post-hoc or unplanned analyses will be clearly identified in the respective study analysis report.

The structure and content of this document provides sufficient detail to meet the requirements identified by the International Conference on Harmonisation (ICH) and the PCTU SOP (PCTU/07).

The following were reviewed in preparation for writing this document:

Trial protocol version 7 24/06/2011

ICH E9 Guidance on statistical principals for clinical trials

ICH E3 Structure and content of clinical study reports

CONSORT guidelines for the reporting of randomised trials

PCTU_DM_04 Standard Operating Procedures (SOP) for: Data Entry, Quality Control, Data Extraction and Database lock

Members of the writing committee

Clare Rutterford (CR) was primarily responsible for (i) writing the Statistical Analysis Strategy and (ii) writing the computer code implementing the analysis strategy and (iii) implementing the strategy at the point of analysis all under the guidance of Professor Sandra Eldridge (SE).

This document has been developed prior to examination of trial data and will not be implemented prior to final approval and after the database has been locked to changes.

Summary

Changes from planned analysis in the protocol

- During November 2011 eleven WAIT participants were randomised not in accordance with the predefined schedule. The DMC recommended the inclusion of these 11 incorrectly randomized participants in the analysis and a sensitivity analysis without them included.
- Five participants were randomised with the incorrect genotype recorded at stratification and will be analysed as randomised.
- One participant AB161 was randomised and allocated a box of IMP; however they did not receive the medication and were then found to be ineligible. They shall be excluded from the analysis
- A couple of children received the wrong box of medication during the trial (approximately three doses). They shall be analysed as randomised
- A handful of participants were withdrawn prior to receiving study medication. Their study medication was reallocated to future participants. CR expressed concern whether this affected the allocation schedule that may distort the balance of the Active/Placebo blocks. Consensus was that the numbers were small so any effect will be negligible and the participants should be analysed as randomised.

STUDY OBJECTIVES AND ENDPOINTS

Study objectives

Primary objectives

1.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for unscheduled medical attention (GP visit, hospital attendance, hospital admission) for wheeze.

Secondary objectives

- 2.To determine whether the effect of treatment on the primary analysis is different depending upon ALOX5 status (5/5 vs. 5/x and x/x).
- 3.To determine whether intermittent treatment with oral montelukast in preschool children reduces the time to first medical attendance.
- 4.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for each type of medical attention for wheeze: hospital admissions; hospital attendance; and GP visits.
- 5.To determine whether intermittent treatment with oral montelukast in preschool children reduces the time to first occurrence of each type of medical attention for wheeze: hospital admissions; hospital attendance; and GP visits.
- 6.To determine whether intermittent treatment with oral montelukast in preschool children reduces the duration of hospital admissions.
- 7.To determine whether intermittent treatment with oral montelukast in preschool children reduces the number of episodes, duration and time to first event of wheeze and cold.
- 8.To determine whether intermittent treatment with oral montelukast in preschool children reduces the need for alternative medications (Steroids, Salbutamol).
- 9.To describe the safety profile of montelukast.
- 10.To describe parents opinion of treatment efficacy
- 11.To describe compliance to medication
- 12.To determine whether baseline urinary eicosanoid level is different across baseline groups: ALOX5 status (A or B), leukotriene genes and, type of wheeze (episodic, multitrigger). **NOTE ANALYSIS DETAIL NOT CONTAINED IN THIS PLAN**
- 13.To determine whether montelukast is cost effective. **NOTE ANALYSIS DETAIL NOT CONTAINED IN THIS PLAN**

Exploratory objectives

- 14.To determine whether the effect of treatment on the primary analysis is different depending upon ALOX5 status (categorised as (5/5 vs. 5/x) vs. x/x).

Outcome measures

Primary outcomes

The number of times a child attends for an unscheduled medical opinion (a summation of hospital admissions, attendances, GP visits,) with respiratory problems over a 12 month period as confirmed from clinical records

Secondary outcomes

Breakdown of unscheduled medical opinion

Hospital admissions:

- Number of hospital admissions over the 12 month period as recorded at each phone call
- Duration of hospital admissions as recorded at each phone call
- Time from randomisation date to date of first hospital admission as recorded at each phone call

Hospital admission for wheeze:

- Number of hospital admissions over the 12 month period as recorded at each phone call
- Time from randomisation date to date of first hospital admission as recorded at each phone call

Hospital attendance for wheeze:

- Number of hospital attendances (A&E) over the 12 month period as recorded at each phone call
- Time from randomisation date to date of first hospital attendance (A&E) as recorded at each phone call

Unscheduled GP visit for wheeze:

- Number of unscheduled GP visits over the 12 month period as recorded at each phone call
- Time from randomisation date to date of first unscheduled GP visit as recorded at each phone call

Description of wheezing episodes

Wheeze:

- Number of wheeze episodes* as recorded on the diary card
- Time to first episode* of wheeze as recorded on the diary card
- Duration of wheeze episodes* as recorded on the diary card

Cold

- Number of cold episodes* as recorded on the diary card
- Time to first episode* of cold as recorded on the diary card
- Duration of cold episodes* as recorded on the diary card

***Definition of episode of wheeze and cold: The duration of an episode is defined as the days from the start of symptoms until the last days of symptoms (includes both start and stop day) followed by a period of 5 symptom free days.**

Medication use

Steroids (OCS):

- The number of courses per year (and total number of days) as recorded on the diary card. Each mention of use on a separate diary card indicates a course.
- The proportion receiving none vs. any during the trial as recorded on the diary card or in the phonecall data.

Steroids (ICS):

- Proportion starting ICS during the trial as recorded on the diary card or phonecall data (baseline data (T2) indicates whether child was on ICS at the start of the trial)

Salbutamol:

- Total number of puffs overall per episode of wheeze as recorded on the diary card
- Total number of puffs (Salbutamol use per year)

Investigational Medicinal Product (IMP) usage:

- The number of IMP initiations (whether for wheeze or cold).
- Mean sachets (IMP use) per episode (wheeze or cold) as recorded on the diary card
- Compliance calculated from diary card, number dispensed and number returned

Inflammatory outcomes

- Baseline and exit urinary eicosanoid level
- Leukotriene genes (approximately 150 genes)

Note: this data is not stored on the main trial database and the analysis is not included within this plan

Safety outcomes

- The number of withdrawals from the trial per group
- Serious adverse events per group
- Adverse events per group
- All cause mortality per group
- Mortality due to exacerbation of asthma per group
- Mortality due to respiratory infection per group

Economic outcomes

Costs due to wheeze:

Unit costs will be assigned for the cost of medical attendances, medicines and time off work. The analysis of economic and qualitative outcomes is not contained within this analysis plan.

STUDY METHODS

Overall study design and plan

Target for randomisation: 650 intervention and 650 control participants

Date of first randomisation: 25/10/2010

Date of last randomisation: 27/12/2012

Trial design: Individually randomized, parallel group

Blinding: Participants and their treating clinician are blind to treatment allocation

Randomised Interventions: Montelukast vs. placebo

Allocation ratio: 1:1

Selection of study population

Inclusion Criteria

- age ≥ 10 months and ≤ 5 years on the day of consent.
- 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
- parent or guardian able to give written informed consent for their child to participate in the study.

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 or sickle cell disease
- 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.

Method of treatment assignment and randomisation

Randomisation was stratified according to ALOX5 promoter polymorphism status. This yielded 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.

Children (participants) in each of these two genotype groups were assigned consecutive randomisation numbers from randomised permuted blocks of 10. Within each block equal numbers of children were randomly allocated to placebo and active treatment. When all numbers from the first block had been assigned a new block of randomisation numbers was allocated to that genotype group, until a total of 1300 children in groups 1 and 2 combined had been assigned a randomisation number. If a randomisation number was assigned to a child who did not subsequently take any dose of IMP, the IMP bearing that randomisation number was returned to pharmacy, and the randomisation number may have been assigned to another child (participant).

Treatment masking (Blinding)

This was a double-blind trial: neither subject nor investigator was aware of a subject's allocation. Active and placebo batches of IMP had identical packaging, labelling and appearance.

Sample size determination

This trial is powered to detect a clinically significant difference in the number of attacks of wheeze between intervention and control arms. We also had power to detect large differences 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.

Data on mean (0.76) and standard deviation (1.22) of number of attacks come from 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.

DATA COLLECTION

Baseline

Demographics

Height in cm

Weight in Kg

Age in years

Sex (Male; Female)

Stratum (A or B)

Ethnicity (Asian or Asian British; Mixed; Black or Black British; White; Other)

Risk factors: Birth, Atopy and Family History (Yes, No)

Preterm birth <37 wk gestation; Birth weight <2500g; Food allergy; Drug allergy; itchy rash for >6 months; Eczema; Tobacco exposure in utero; Tobacco exposure in household; daycare attendance; immunisation status for Pneumococcus; immunisation status for influenza; history of asthma mother; history of asthma father.

Pre-study illness and therapy (Yes/No)

Episodic wheeze; multitrigger wheeze; admitted to hospital in last year; ever admitted to hospital; Preventer therapy none; Preventer therapy antileukotriene; Preventer therapy Maintenance inhaled steroids; Preventer therapy episodic inhaled steroids

Age at first wheeze in months

Interval between onset of URTI and wheezing (hours)

Number of courses of systemic steroids in the last year

Number of unscheduled medical attendances for wheeze in last year

Pre-existing conditions

Medical condition

Date of diagnosis

Resolved/ongoing

Current treatment

Follow up

Unscheduled medical attendance

Phone call data: Type of attendance (A&E; Hospital; GP; Pharmacist; Other)

Phone call data: Duration of visit (calculated from date of admission and date of discharge)

Description of wheezing episodes

Diary card: Wheeze in the last 24 hours (Yes/No)

Diary card: Date of diary card entry

Diary card: Duration of wheeze episodes will be calculated where wheeze in the last 24 hours has been ticked over consecutive days

Diary card: Total duration of wheeze days over follow-up period

Medication use Steroids (OCS)

Diary card: Date
 Diary card: Medication (where medication includes Prednisolone and its variations)
 Diary card: Dose
 Diary card: Units
 Diary card: Days
 Diary card: Doses per day
 Phone call data: Other medications used (where medication includes Prednisolone and its variations)

Medication use Steroids (ICS)

Diary card: Date
 Diary card: Medication
 Diary card: Dose
 Diary card: Units
 Diary card: Days
 Diary card: Doses per day
 Phone call data: Other medications used

Medication use Salbutamol

Diary card: Date
 Diary card: blue inhaler used today?
 Diary card: How many times blue inhaler used?
 Diary card: How many puffs when blue inhaler used?
 Phone call data: Other medications used (where medication includes salbutamol and its variations)

Medication use IMP

Diary card: Date
 Diary card: Wheeze in last 24 hours (Yes/No)
 Diary card: Cold in last 24 hours (Yes/No)
 Diary card: Trial medicine used today (Yes/No)
 Phone call data: Number of IMP initiations
 Phone call data: Total days used

Adverse events and serious adverse events

Clinical AE term (categorised as; minor injury, GI, URTI, CNS, minor infection, allergy, cutaneous, respiratory, haem)
 SAE term
 SAE expected (Yes/No)
 Start date
 End date
 Date of death
 Duration in hours
 Intensity (Mild, Moderate, Severe)
 Action taken (none, interrupted, discontinued, reduced)
 Related to study drug (Definitely not, probably not, possibly, probably, definitely)
 SAE resolved (resolved, resolved with sequelae)
 Sequelae details
 Outcome (improved, persisting, worsened, fatal, unknown)

Withdrawals

Withdrawal (from treatment or trial)
 Date of withdrawal
 Reason for withdrawal (eligibility no longer met, death of participant, other adverse event, deterioration of pre-existing condition, Poor adherence to treatment, Perceived lack of efficacy, unable to locate participant, other)
 Withdrawal decision by (CI, PI, Referring investigator, Carer, Participant, other)
 Permission to use data (do not use any data, use partial data up to withdrawal, use all data up to withdrawal, collect and use all follow up data)

Code broken (Yes/No)

Timing of data collection

Each child (participant) was followed up for 12 months post randomisation with data collection taking place at 2, 4, 6, 8, 10 and 12 months.

GENERAL ISSUES FOR STATISTICAL ANALYSIS

All analyses will be conducted two sided and significance interpreted at the 5% significance level.

Blinding of the statistical analysis

The statistical analysis will be conducted unblinded so that the appropriate treatment code can be used in the models fitted.

Analysis populations

Intent-to-treat population

The intention-to-treat (ITT) sample is defined for this trial as all participants randomized into the trial included in the intervention group to which they were randomised.

Available-case population

The available Case (AC) sample is defined for this trial as all participants randomized into the trial included in the intervention group to which they were randomised where outcome data are available.

Per protocol population

The Per Protocol (PP) sample is defined as the available case sample with those participants who discontinue IMP or were randomised incorrectly being excluded.

Safety population

The safety population includes all participants.

Other populations

Two populations are described for the sensitivity analyses described in section 8.5. The first is based on the ITT population replacing any stratification factors that were incorrectly defined at randomisation with the corrected values. The second is based on the ITT population with the exclusion of 11 incorrectly randomised participants.

Database

Description

The data were entered into and stored in a Microsoft Access database. Data were entered by trial staff who were blind to treatment group.

Data quality

Source data verification is performed for 10% of CRFs by the trial team.

Database freeze and lock

Once the trial team have completed all data entry and checking. The statistician responsible for the analysis will conduct or oversee additional data checks. These include things such as range checks, logical and consistency checks which may not be picked up by checks performed at the individual level. Procedures implemented to database lock will be followed in accordance with the relevant SOP (PCTU_DM_04 Standard Operating Procedures (SOP) for: Data Entry, Quality Control, Data Extraction and Database lock)

Analysis will take place when the database is considered final.

Analysis software

The analysis will be carried out using Stata version 12.0.

Methods for withdrawals, loss to follow-up and missing data

Those participants who withdraw and provide permission to use their data will be included in the analysis up to the point of withdrawal.

For the primary outcome phonecall data, at the time of writing (prior to unblinding) we have:

Full 12 months data on 1134/1347(84%)

29/1347 (2%) participants withdrew before the first 2 month phonecall and have no data collected as expected

12/1347 (0.9%) do not have any follow up data and this is being queried with the sites

Partial follow up data is available for 172 (13%). 44 of these participants did not formally withdraw from follow up. This is being queried with the sites

After data cleaning we expect the levels of missing data to improve. Due to these relatively low levels of missing data, and that the follow up time for each participant is to be included in the analysis no imputation of the missing data will be performed

Method for handling centre effects

We do not anticipate there to be any affect of centre and this will not be adjusted for in the analysis

Method for handling randomisation stratification or minimisation factors

The randomisation was stratified by genotype and this will be included as a covariate in all analyses.

Method for handling clustering effects

Some outcomes are collected at the level of episode, (duration of wheeze episode, duration of cold episode, duration of hospital admission) therefore we have episode data within children. In these cases a random effect is included for child.

Method for selecting other variables that will be adjusted for

All analysis will only be adjusted for genotype (see section 2.7).

Multiple comparisons and multiplicity

No formal method will be used to account for multiple comparisons. All comparisons will be defined within this document *a-priori* and all will be reported.

Method for handling non-adherence

Analysis of all primary and secondary outcomes will be performed on an intention-to-treat basis. A Complier Average Causal Effect (CACE) analysis and per protocol analysis will also be conducted for the primary analysis.

Method for handling time-varying interventions

Not applicable

Method for handling outliers and influential points

Where any outliers are identified they will be investigated to determine whether they are true recorded values or a data entry error. Where outliers are identified as a true recorded value, an assessment will be made as to whether there are clear quality indications to remove them. If such indications exist, the outliers will be removed. If such indications do not exist, the analysis will be performed both including and excluding the outlier to assess the robustness of the conclusions.

Data from external sources

Not applicable

Derived and computed variables

All derived and computed variables will be documented in the analysis programmes. The primary outcome is a summation of all types of medical attendances across the entire trial, for each participant.

The primary outcome, and the breakdown of unscheduled medical opinion, will be taken solely from the phone call data as this data has been confirmed against clinical records.

Medication use data may be recorded on either the phone call CRF and/or the diary card. A medication will be defined as being used if it appears in either of these two records.

Medical attendance data was collected strictly within 12 months, as calculated from the date of randomisation. Participants who do not experience an event are censored at exactly 12 months of follow up or the point of withdrawal from follow up. Any diary data collected outside of the 12 month follow up will be excluded from the analysis. Participants who do not experience episodes of cold or wheeze will be censored at the point of 12 months from randomisation or withdrawal from medication, as diary cards are not completed for those not taking IMP.

DESCRIPTIVE ANALYSES

The proposed tables to be populated during the analysis can be found in the appendix

Participant flow

Participant throughput will be summarized in a CONSORT diagram.

Representativeness of sample

Information unavailable to make this comparison

Baseline comparability of randomised groups

See table 1 in the appendix for the variables to be used in these comparisons.

Demographics**Prior and concurrent medications****Baseline and screening conditions****Baseline medical history****Baseline physical exam****Cluster characteristics if cluster randomised****Characteristics of care providers where applicable****Comparison of losses to follow-up**

See table 2 in the appendix

Comparison of compliance to treatment and protocol

Compliance to treatment will be summarised as the number of returned used sachets of medication.

Emergency or accidental unblinding of randomised treatment

All unblindings will be summarised by treatment group

INTERIM ANALYSES AND SAFETY MONITORING ANALYSES**Purpose of interim analyses**

No interim analyses of the data were planned or conducted.

Monitoring plan

A Data Monitoring Committee was initiated at the beginning of the study. This committee met three times during the course of the study and saw accumulating data by treatment group on recruitment, safety and efficacy. All data was presented descriptively with no hypothesis testing.

Stopping rules

Not applicable

Measures taken to minimize bias

Not applicable

Adjustment for p-values

Not applicable

Interim analysis for sample size adjustment

Not applicable

ANALYSIS OF PRIMARY OUTCOME**Definition of outcome measure**

The primary outcome for each participant is the total number of unscheduled medical attendances over the course of the trial.

Descriptive statistics for outcome measure

The primary outcome will be summarised for each treatment group as the total number of events and corresponding median length of follow up time per treatment group.

Data will be presented as mean (sd) or median (interquartile range) depending upon the distribution of the data.

Primary analysis

The primary analysis will be a Poisson regression model with the follow up time of each individual fitted as an exposure variable and with a random effect for individual to account for overdispersion.

The incident rate ratio (IRR) for the treatment effect and corresponding 95% confidence interval will be presented. An IRR of less than 1 indicates a benefit of Montelukast in reducing the rate of unscheduled medical attendance needed.

Assumption checks and actions to be taken if assumptions do not hold

The fit of the model will be compared to a model without a random effect using the likelihood ratio test, and the fit will be assessed using diagnostic plots (residuals versus fitted values), alternative distributions to the Poisson such as the Negative binomial or removal of the random effect shall be considered where necessary for improved fit.

Other analysis supporting the primary (inc. sensitivity analyses)

The primary analysis will be performed on the per-protocol population and using a CACE analysis.

It will be repeated replacing any stratification factors that were incorrectly defined at randomisation with the corrected values (see section 1.4).

It will be repeated with exclusion of 11 incorrectly randomised participants (see section 1.4).

ANALYSIS OF SECONDARY OUTCOMES

Definition of outcome measure

individual type of medical attendance: (hospital admission, hospital attendance (a&e), and GP visit)

Duration (in days) of hospital admission

Number of wheeze episodes

Total duration of wheeze episode

The number of steroid (OCS) courses per year

The number of IMP courses per year

first hospital admission

first hospital attendance (A&E)

first GP visit

first episode of wheeze

proportion receiving no steroids (OCS) vs. any during the trial

Proportion starting steroids (ICS) during the trial

Salbutamol use per year

Salbutamol use per episode of wheeze per year

Descriptive statistics for outcome measure

Each outcome will be summarised for each treatment group as the total number of events or average duration of episode.

Data will be presented as mean (sd) or median (interquartile range) depending upon the distribution of the data.

Secondary analysis

The primary analysis will be repeated for each of the following secondary outcomes:

individual type of medical attendance: (hospital admission, hospital attendance (a&e), and GP visit)

Duration (in days) of hospital admission

Number of wheeze episodes

Duration of wheeze episode

The number of steroid (OCS) courses per year

The number of IMP courses per year

Time to event data will be summarised using Kaplan Meier plots. The treatment effect will be evaluated using a Cox regression model. The Hazard Ratio (HR) for the treatment effect and corresponding 95% confidence interval will be presented. A

HR of less than 1 indicates a benefit of Montelukast in reducing the time to first event.

first hospital admission

first hospital attendance (A&E)

first GP visit

first episode of wheeze

Binary outcomes will be analysed with logistic regression

proportion receiving no steroids (OCS) vs. any during the trial

Proportion starting steroids (ICS) during the trial

Assumption checks and actions to be taken is assumptions do not hold

The assumption of proportional hazards for the cox regression model will be checked using the methods proposed by Grambsch and Therneau¹⁹. If this assumption is violated, alternative methods will be used.

See section 8.4 for Poisson regression assumption checks.

Other analysis supporting the secondary (inc. sensitivity analyses)

None

SAFETY AND TOLERABILITY ANALYSES

Adverse event data will be summarised with descriptive statistics.

Intervention exposure

The number of participants receiving medication will be summarised per treatment group.

All Adverse events

See table 7 in the appendix

Adverse events leading to withdrawal

See table 2 in the appendix

Serious adverse events

See table 8 in the appendix

Clinical laboratory evaluations

There are no AEs defined by laboratory evaluations

SUBGROUP ANALYSES

Definition of outcome measure

For each participant, the total number of unscheduled medical attendances over the course of the trial.

Definition of subgroups

The primary analysis will be repeated to assess whether there is a differential effect of treatment by:

Genotype, categorised as 5/5 vs (5/x and x/x) and alternatively as (5/5 and 5/x) vs x/x

Whether ICS taken at baseline (yes, No)

Episodic vs multitrigger wheeze at baseline

Sample size justification for the subgroup analysis

The study has been powered to detect a specific interaction effect.

Descriptive analysis for subgroups

The mean and standard deviation of the number of unscheduled medical attendances will be summarised for each ALOX5 genotype and each treatment group

Method of analysis

The primary analysis will be repeated including an interaction term between treatment and stratum. The significance of the interaction term assessed.

AMENDMENTS TO VERSION X

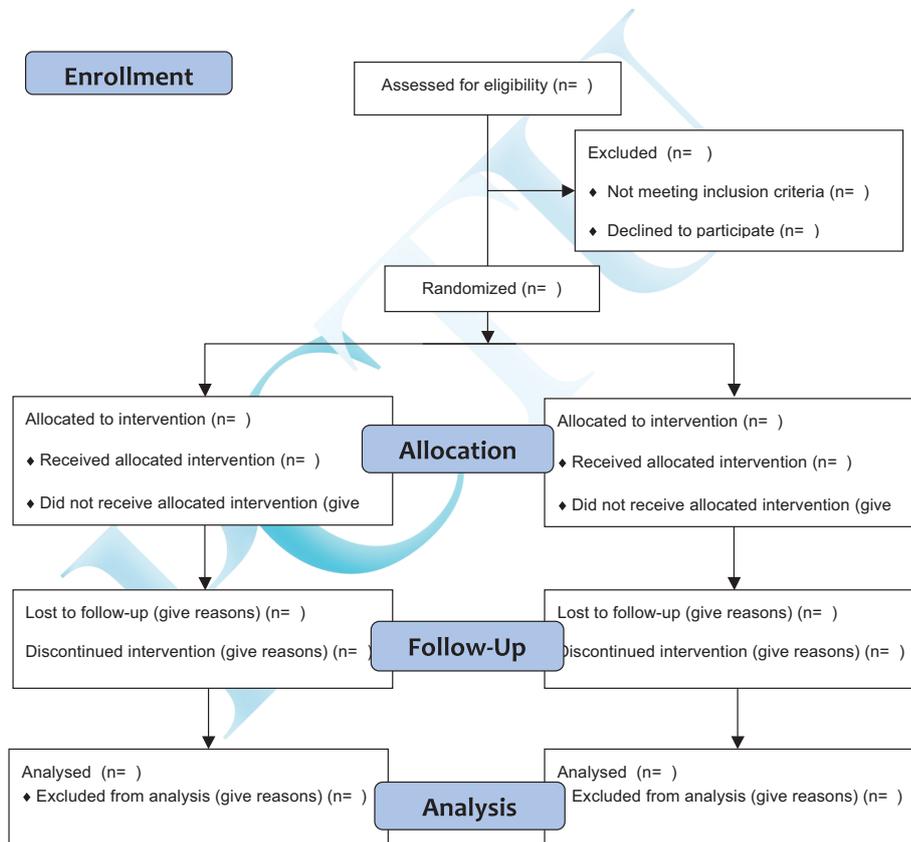
REFERENCES

APPENDICES

This document was created based on the Mental Health and Neuroscience Clinical Trials Unit (MH&N CTU) analysis strategy template (version 1.5;13/02/2008)

Appendix: Statistical Analysis Report Template

CONSORT Flow Diagram



PCTU_SOP SP_01 Associated document

Not to be used without prior permission by the PCTU.

Version 2.0

Table 1: Baseline comparability of treatment groups

ITT population	Montelukast			Placebo		
	5/5	5/x and x/x	N= Total	5/5	5/x and x/x	N= Total
Height (cm)						
Weight (Kg)						
Age (years)						
Gender						
		Male				
		Female				
Stratum						
		A				
		B				
Ethnicity						
		Asian or Asian British				
		Mixed				
		Black or Black British				
		White				
		Other				
Pre existing conditions						
		X				
		Y				
		Z				
Age at first wheeze (months)						
Interval between onset of URTI and wheezing (hours)						
Episodic wheeze						
Multi-trigger wheeze						
Admitted to hospital in last year						
Admitted to hospital ever						
Preventer therapy						
		None				
		Antileukotriene				
		Maintenance inhaled steroids				
		Episodic inhaled steroids				
Risk factors						
		Preterm birth <37 wk gestation				
		Birth weight <2500g				
		Food allergy				
		Drug allergy				
		Itchy rash for > 6 months				
		Eczema				
		Tobacco exposure in utero				
		Tobacco exposure in				

household
 Daycare attendance
 Immunised for
 Pneumococcus
 Immunised for influenza
 History of asthma in
 mother
 History of asthma in
 father
 Numbers are N(%) or mean (SD)

This table is to be repeated for the alternative stratification (5/5 and 5/x) versus (x/x).

Table 2: losses to follow up

ITT population	Montelukast			Placebo		
	Trial	N= Treatment	Total	Trial	N= Treatment	Total
Reason for withdrawal						
Eligibility no longer met						
Death						
Other adverse event						
Deterioration of pre-existing condition						
Poor adherence to treatment						
Perceived lack of efficacy						
Unable to locate participant						
Other						
Decision made by						
CI						
PI						
Referring investigator						
Carer						
Participant						
Other						
Permission to use data						
Do not use any data						
Use partial data up to withdrawal						
Use all data up to withdrawal						
Collect and use all follow up data						
Numbers are N (%)						

Table 3 Primary analysis: unscheduled medical attendances for wheeze over 12 months

ITT population	Montelukast N=	Placebo N=	Adjusted IRR (95% CI) ¹	p-value
Follow up time (days)				
Number of :				
Any medical attendance				
	All			
	Straum A			
	Stratum B			
	Stratum 5/5 and 5/x			
	Stratum D x/x			

ICS at baseline
Multitrigger vs episodic wheeze

Hospital admissions
Hospital attendances (A&E or admission)
Unscheduled GP visits

Parents considered medication to be efficacious, N(%)

Data are mean (SD)

¹ Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual to account for overdispersion with follow up time fitted as the exposure. An interaction term has been included to assess whether there is a differential treatment effect dependent on stratum.

Table 4: Episodes of cold and wheeze

ITT population	Montelukast N=	Placebo N=	IRR (95% CI) ¹	p-value
Number of:				
Wheeze episodes				
Cold episodes				
Days wheezing				
Returned used medication sachets				
Duration of:				
Wheeze episodes (days)				
Hospital admission (days)				

Data are mean (SD)

¹ Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual to account for overdispersion with follow up time fitted as the exposure. Duration of each hospital admission is analysed using Poisson regression with fixed effects for stratification factor and treatment group a random effect for individual with follow up time fitted as the exposure.

Table 5: Time to first event of unscheduled medical attendance, wheeze or cold

ITT population	Montelukast N=	Placebo N=	HR (95% CI)	p-value
Time (in days) to first:				
Hospital admission				
Hospital attendance (A&E or admission)				
Unscheduled GP visit				
Episode of wheeze				
Episode of a cold				

Data are median (IQR)

Data are analysed using a Cox regression model with fixed effects for stratification factor and treatment group

Table 6: Medication usage

ITT population	Montelukast N=	Placebo N=	IRR or OR (95% CI)	p-value
Steroids (OCS)				
¹ Number of courses, mean (SD)				
² Proportion receiving OCS, N (%)				
Steroids (ICS)				
² Proportion starting, N (%)				
Salbutamol				
¹ Number of puffs used per episode, mean(SD)				
Total puffs used per year				
Investigational Medicinal Product				
¹ Number of initiations, mean (SD)				
¹ Number of sachets per episode, mean (SD)				
Number of sachets used per year				
¹ Data are analysed using Poisson regression with fixed effects for stratification factor and treatment group and a random effect for individual to account for overdispersion with follow up time fitted as the exposure.				
² Data are analysed using logistic regression with fixed effects for stratification factor and treatment group				

Table 7 Total adverse events per group

Safety population	Montelukast N=	Placebo N=
All events		
	Minor injury	
	GI	
	URTI	
	CNS	
	Minor infection	
	Allergy	
	Cutaneous	
	Respiratory	
	Haem	
Possibly, probably or definitely related		
	Minor injury	
	GI	
	URTI	
	CNS	
	Minor infection	
	Allergy	
	Cutaneous	
	Respiratory	
	Haem	
Data are n (%)		

Table 8: Serious Adverse events per group

Safety population	Montelukast N=	Placebo N=
Death		
XXX		
XXX		
...		
Data are n (%)		

Appendix 6 Protocol amendments

The study underwent a number of protocol and other amendments. Where an amendment has not been subject to ethics committee approval or MHRA scrutiny it has been deemed non-substantial by the sponsor. These amendments are summarised in the list below and detailed in *Table 13*.

1. Change to meet initial ethics committee conditions (before study commenced).
 - PIS update to include dosage, duration, side effect, treatment duration, action if vomited, overdosage outcomes.
 - update regarding parent–researcher contact time
 - ‘What will happen when I initiate therapy section?’ added
 - PIS states travel expenses will be covered
 - statement regarding inefficacy of steroids removed
 - typographical errors corrected
 - informed consent form has checkboxes added
 - advice to parents regarding contacting their insurers added
 - update to say that lay summary of findings will be offered.
2. Change to allow specific tests in Aberdeen [exhaled breath condensate (EBC), lung function (LF), skin prick test (SPT), fractional exhaled nitric oxide (FeNO) – never performed].
3. Diary card changes designated as minor amendment.
4. Permission to repackage medicines into smaller boxes because of reduced supply.
5. Invitation sheet amended with ‘or has been prescribed meds for wheeze’ to explain why child has been identified as a possible participant.
6. Multiple new site additions.
7. Removal of DSMC charter from protocol.
8. Removal of Aberdeen extra tests from protocol (these were never performed).
9. Addition of cover letters for primary care and hospital identified patients.
10. PIS amended to state that montelukast is not a new/experimental drug.
11. Addition of a recruitment poster.
12. Addition of a GP invitation letter.
13. Update to GP recruited/not-recruited letters (tidier format, reference to website).
14. Update to allow medications sourced from outside the European Union (EU).
15. Update to introduce parent reminder sheet (an aide-memoire)
16. Update to allow Novalabs to have a primary packaging role.
17. Final protocol update:
 - to reflect multicentre nature of trial (especially pharmacy)
 - to allow 24 hours or an overnight stay for parental consideration of the PIS
 - removal of reference to weighing salbutamol canisters
 - to allow second urine specimens to be collected in other sites as well as London
 - to clarify status of viral wheezing episodes as not being AEs
 - remove ambiguity re: need for parents to contact trial team when starting medicine
 - remove reference to Pragmatic Clinical Trials Unit (PCTU) in the PIS.
18. Addition of failed contact letters.

TABLE 13 Study amendments

Amendment number	Amendment Type	Description of protocol version change or amendment	Date submitted to REC	Date of approval	New detailed protocol version date
1	Major	Initial protocol submission to REC	1 October 2009	23 November 2009	Version 1; 1 October 2009
N/A	N/A	Internal minor revisions – not submitted	N/A	N/A	Version 2–3; 23 April 2012 to 11 September 2010
2	N/A	Update to meet initial REC-imposed approval conditions	12 September 2010 (from cover letter)	25/10/10 (received dates, no approval date given)	Version 4; 7 June 2010
3	Major	Amendment to allow additional tests in Aberdeen	13 October 2010 (from cover letter)	27 October 2010 (not 27 July 2010 as per REC letter)	Version 5; 26 July 2010
4	Minor	Diary card minor amendment	N/A	Internal	
5	Minor	MHRA NSA to split boxes	11 March 2011	11 March	
	MHRA				
6	Minor	NSA parent invitation sheet version 3	30 March 2011	5 April 2011	
7	Major	<ol style="list-style-type: none"> 1. New sites, principal investigators, local collaborators (PCT) added to protocol 2. Research nurses, statistician details added to protocol 3. DSMC charter removed from protocol for clarity – stored separately in TMF 4. GP cover letter to parent (with reminder letter) added to improve primary care recruitment and protect patient information 5. Aberdeen amendment (protocol version 5) removed from protocol 6. Hospital attendee cover letter added to clarify information sheet 7. The text 'Montelukast is not a new medicine and has been licensed as safe for use in young children for several years.' added to the PLS, version 2 	11 March 2011	14 April 2011	Version 6; 10 March 2011

Amendment identifiers		Description of protocol version change or amendment	Date submitted to REC	Date of approval	New detailed protocol version date
Amendment number	Type				
8	Major	<ul style="list-style-type: none"> • WAIT GP letter, version 1, 8 October 2009 → WAIT GP letter, version 2, 13 April 2011 • GP invitation letter, version 1, 13 April 2011 • WAIT poster, version 1, 13 April 2011 • (REC letter says 8 October 2011 – which is in the future and hence erroneous) 	13 April 2011	28 April 2011	
9	Major MHRA	Amendment to allow use non-EU sourced IMP	14 April 2011	6 May 2011	
10	Major	New sites and parent reminder sheet, version 1	1 June 2011	22 June 2011	
11	Major	New sites	24 June 2011	12 July 2011	
12	Major	To allow Novalabs to package unsacheted raw material from manufacturer	30 June 2011	13 July 2011	
13	MHRA Major	Replacement of protocol version 6 with version 7. This updates the protocol to reflect: <ul style="list-style-type: none"> • Additional trial sites • Updated medicines distribution and replacement procedure – to allow dispensing from local sites and account for varying date and need for replacement • Update timing of interview to reflect convenience of subject being paramount • Update to allow 24 hours or an overnight stay as minimum duration for consideration of trial information before consent to enter trial – to account for occasional lack of documentation of timing of initial approach, and also to allow recruitment following an overnight admission rather than enforcing a home visit or parental clinic visit. This suggestion has met with approval from parents 	24 June 2011	17 August 2011	

continued

TABLE 13 Study amendments (continued)

Amendment identifiers		Description of protocol version change or amendment	Date submitted to REC	Date of approval	New detailed protocol version date
Amendment number	Type				
		<ul style="list-style-type: none"> Update to remove reference to weighing of salbutamol canisters. Usage is estimated from parental reporting in diary cards. This change was recommended by the trial steering committee as weighing canisters was deemed impractical Update to suggest second urine specimens (during illness) be collected at all sites rather than solely the Royal London Hospital Update to specify that viral and wheezing episodes that trigger IMP use not be deemed as AEs, as this temporal association is dictated by the protocol instructions to parents (they are told to use the IMP when their child has a cold or wheeze) Replacement of 'Parent Reminder Sheet v1 250511.doc' with 'Parent reminder sheet v2 240611.doc'. This removes the erroneous reference to parents contacting trial team when starting medicine Replacement of 'Parent Information Sheet v2 290311.doc' with 'Parentinformationsheet-version3 220611.doc'. This corrects some misinformation regarding trial procedure (details in 1a-g and in tracked changes version). In particular, references to the clinical trials unit are removed as they are not actually involved in the study Addition of new sites with associated PIs to the study protocol as an appendix (this forms a separate amendment as discussed with Sophie Vella) Addition of documents: 'Wait failed contact letter – general v1 130611.doc' and 'Wait failed contact letter – medicines v1 130611.doc' for use when telephone contact proves impossible 			

Amendment identifiers		Description of protocol version change or amendment	Date submitted to REC	Date of approval	New detailed protocol version date
Amendment number	Type				
14	Major	We would like to request approval for the addition of a consent form to allow the use of patient data on children who are not recruited to the study. This will allow us to ascertain the characteristics of those who are not recruited as well as those who are	22 July 2011	Rejected 17 August 2011	
15	Version 7	New sites	29 July 2011	4 August 2011	Version 7; 24 June 2011
16	Major	PIs, version 3 → version 4 to explicitly state that specimens will be sent to London	24 August 2011	26 October 2011	
		To state that a copy of the information will be stored in London			
		To modify consent form for unrecruited patient data retention (see 14)			
17	Minor	Modification to make WAIT trial clinic letter version 2 → version 2.1 (generalisable)	3 October 2011	1 November 2011	
18	Major	Close out letter and certificate of thanks	23 September 2011	26 October 2011	
19	Minor	Minor modifications diary card, version 3 → version 4	24 January 2012	22 June 2012	
20	Major	New sites (Hinchingsbrooke, Kilmarnock, etc.)	6 March 2012 (note: REC letter stated erroneous receipt date due to revised submission)	7 March 2012	
21	Major	Additional PI (Chester)	9 March 2012 (REC letter says 20 March 2012)	4 April 2012	
22	Major	New sites (Huddersfield, Bradford)	22 June 2012	6 July 2012	

continued

TABLE 13 Study amendments (continued)

Amendment identifiers		Description of protocol version change or amendment	Date submitted to REC	Date of approval	New detailed protocol version date
Amendment number	Type				
23	Minor	Study extension	20 June 2012	21 June 2012	
24	Major	Protocol, version 8 – to allow repeat urines/testing for urinary cotinine	27 June 2012	17 July 2012	Version 8; 20 June 2012
25	Major	New sites (Luton, Airedale, Warrington, Durham)	16 July 2012 (REC letter says 24 June 2012)	15 August 2012	
26	Major	Change of PI – Royal Berkshire	2 April 2012	4 April 2012	

N/A, not applicable; NSA, non-substantial amendment; PCT, primary care trust; REC, Research Ethics Committee; TMF, trial management file.

Appendix 7 Ethics committee and regulatory approvals

Copy of Medicines and Healthcare products Regulatory Agency approval

Safeguarding public health



Dr J Grigg
BARTS AND THE LONDON NHS TRUST
2 NEWARK STREET
LONDON
E1 2AT
UNITED KINGDOM

22/02/2010

Dear Dr J Grigg

THE MEDICINES FOR HUMAN USE (CLINICAL TRIALS) REGULATIONS 2004 S.I. 2004/1031

Our Reference: 21313/0024/001-0001
Eudract Number: 2009-015626-11
Product: Singulair Paediatric 4mg Granules
Protocol number: 006983 QM

NOTICE OF ACCEPTANCE OF AMENDED REQUEST

I am writing to inform you that the Licensing Authority accepts your amended request for a clinical trial authorisation (CTA), received on 12/02/2010.

The authorisation is effective from the date of this letter although your trial may be suspended or terminated at any time by the Licensing Authority in accordance with regulation 31. You must notify the Licensing Authority within 90 days of the trial ending.

Finally, you are reminded that a favourable opinion from the Ethics Committee is also required before this trial can proceed; changes made as part of your amended request may need to be notified to the Ethics Committee.

Yours sincerely,

**Clinical Trials Unit
MHRA**

Medicines and Healthcare products Regulatory Agency
Market Towers 1 Nine Elms Lane London SW8 5NQ
T 020 7084 2000 F 020 7084 2353 www.mhra.gov.uk

An executive agency of the Department of Health

Copy of ethical approval



National Research Ethics Service
 South East Research Ethics Committee

Telephone: [REDACTED]
 Facsimile: [REDACTED]

23 November 2009

Professor Jonathan Grigg
 [REDACTED]
 [REDACTED]
 [REDACTED]
 [REDACTED]

Dear Professor Grigg

Study Title: Parent-determined oral montelukast therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype.
REC reference number: 09/H1102/110
Protocol number: 1
EudraCT number: 2009-015626-11

The Research Ethics Committee reviewed the above application at the meeting held on 11 November 2009. Thank you for attending to discuss the study.

Ethical opinion

The committee started by commending you on your application.

The committee stated that they had not been provided with the topics that were to be covered in the interview process.

You stated that the interview was covered in the protocol. You went on to say that the questions had been created using feedback from parents.

The committee drew your attention to the PIS and stated that it would need to be amended to contain details regarding dosage, side effects, the length of treatment and the risk of overdose.

You agreed with this and went on to state that it would also cover what would happen if a participant vomited out the drug.

The committee asked about the length of time between the start of treatment and the point at which the researcher would be contacted.

You stated that the parents / guardians of the participant would contact the researcher at the start of treatment and then contact would be made again, 7-10 days after that. Contact would subsequently be made on a monthly basis.

This Research Ethics Committee is an advisory committee to South East Coast Strategic Health Authority
 The National Research Ethics Service (NRES) represents the NRES Directorate within
 the National Patient Safety Agency and Research Ethics Committees in England

The committee suggested that a 'What will happen when I initiate Therapy?' section should be added to the PIS.

You agreed to this.

The committee then asked how it would be judged if the child participant really had a cold.

You stated that this would be down to the parents and suggested that they were experienced and knowledgeable enough to make the judgement. In addition to this, parents would be given training on specific triggers to watch out for.

The committee asked what would happen if the treatment was started when the child participant didn't actually have a cold.

You agreed that this could happen - but assured the committee that it was a safe medication.

The members of the Committee present gave a **favourable ethical opinion** of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. I will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

- a) The PIS should indicate that travel expenses will be covered.
- b) In pt. 1 of the PIS it states that steroid tablets do not work. This statement should be removed.
- c) The PIS should be proofread throughout. There are a few errors that need correcting (for example, the word 'sue' appears instead of the word 'use').
- d) The consent form needs to have boxes inserted so that participants have definite areas to tick.
- e) The PIS needs to advise parents of participants who hold private medical insurance covering the child that they should inform their insurance companies that they are taking part in the trial.
- f) At A53 on the application it states that a lay summary of findings will be offered. In the PIS it states that this summary may be requested. The PIS should be amended to read that the summary will be offered.
- g) The PIS is missing information relating to the possible side effects of the treatment. It also needs to provide information on the length of treatment and the possible risks of overdose.
- h) A 'What will happen when I initiate Therapy?' section should be added to the PIS.

An advisory committee to South East Coast Strategic Health Authority

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at <http://www.rdforum.nhs.uk>. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

Clinical trial authorisation must be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA).

The sponsor is asked to provide the Committee with a copy of the notice from the MHRA, either confirming clinical trial authorisation or giving grounds for non-acceptance, as soon as this is available.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter		19 October 2009
REC application		19 October 2009
Protocol	1	01 October 2009
Investigator CV	Prof Jonathan Grigg	
Participant Information Sheet: Parent (PartI)SWAIT)	1	01 October 2009
Participant Information Sheet: Child	1	01 October 2009
Participant Consent Form: Parent / Guardian (PCFWAIT)	1	01 October 2009
Letter of invitation to participant	1	01 October 2009
GP/Consultant Information Sheets	1	08 October 2009
Evidence of insurance or indemnity		19 October 2009
Sample Diary/Patient Card	1	08 October 2009
Summary of Product Characteristics	SPCmontelukast	12 March 2009
Sponsorship Approval Letter		19 October 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Prof C Katona declared a non-specific interest in this study.

Statement of compliance

This Committee is recognised by the United Kingdom Ethics Committee Authority under the Medicines for Human Use (Clinical Trials) Regulations 2004, and is authorised to carry out

An advisory committee to South East Coast Strategic Health Authority

the ethical review of clinical trials of investigational medicinal products.

The Committee is fully compliant with the Regulations as they relate to ethics committees and the conditions and principles of good clinical practice.

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email

[REDACTED]

09/H1102/110

Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

[REDACTED]

Dr L. Alan Ruben
Chair

Email: [REDACTED]

Enclosures: List of names and professions of members who were present at the meeting and those who submitted written comments "After ethical review – guidance for researchers"

Copy to: Mr Gerry Leonard

An advisory committee to South East Coast Strategic Health Authority

Copy of sponsor approval

**FINAL R&D APPROVAL**

Professor Jonathan Grigg

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Joint Research and Development Office

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

Tel: [REDACTED]
Fax: [REDACTED]9th September 2010

Dear Professor Grigg,

Re: Parent-determined oral Montelukast Therapy for preschool wheeze with stratification for arachidonate-5-lipoxygenase (ALOX5) promoter genotype: WAIT Trial

ReDA Reference: 006539
CSP REF: 35170

I am now happy to inform you that the Joint R&D Office of Barts and The London NHS Trust and Queen Mary, University of London has arranged full indemnity cover for your study against any negligence that might occur during the course of your project.

Please note that all research with an NHS element is subject to the Research Governance Framework for Health and Social Care 2005. If you are unfamiliar with the standards contained in this document, or the BLT and QMUL policies that reinforce them, you can obtain details from the Joint R&D Office, tel [REDACTED] or go to <http://www.dh.gov.uk/PolicyAndGuidance/ResearchAndDevelopment/ResearchAndDevelopmentAZ/ResearchGovernance/fs/en>.

You must stay in touch with the Joint R&D Office during the course of the research project, particularly if/when:

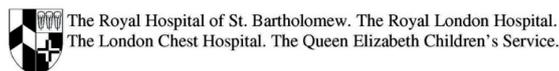
- There is a change of Principal Investigator;
- The project finishes;
- Amendments are made, whether minor or substantial.

This is necessary to ensure that your indemnity cover is valid. Should any Serious Adverse Events (SAEs) occur it is **essential** that you inform the Sponsor within 24 hours. If patients or staff are involved in an incident, you should also contact the Clinical Risk Manager on [REDACTED].

I hope the project goes well, and if you need any help or assistance during its course, please do not hesitate to contact the Office.

Yours sincerely,

Gerry Leonard
Head of Research Resources

**Head of Research Resources:** Gerry Leonard

Appendix 8 Study drugs

This appendix is reproduced with permission from Merck, Sharp & Dohme Ltd. *Singulair Paediatric 4 mg Granules – Summary of Product Characteristics (SPC) – (eMC)*. Medicines.org.uk. 2015.
URL: www.medicines.org.uk/emc/medicine/14071 (accessed 12 November 2015), as compiled by Datapharm:

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- LEGAL CATEGORY

1. NAME OF THE MEDICINAL PRODUCT

SINGULAIR® Paediatric 4 mg Granules

2. QUALITATIVE AND QUANTITATIVE COMPOSITION

One sachet of granules contains montelukast sodium, which is equivalent to 4 mg montelukast. For a full list of excipients, see section 6.1.

3. PHARMACEUTICAL FORM

Granules

White granules

4. CLINICAL PARTICULARS

4.1 Therapeutic indications

SINGULAIR is indicated in the treatment of asthma as add-on therapy in those 6 months to 5 year old patients with mild to moderate persistent asthma who are inadequately controlled on inhaled corticosteroids and in whom “as-needed” short acting β - agonists provide inadequate clinical control of asthma.

SINGULAIR may also be an alternative treatment option to low-dose inhaled corticosteroids for 2 to 5 year old patients with mild persistent asthma who do not have a recent history of serious asthma attacks that required oral corticosteroid use, and who have demonstrated that they are not capable of using inhaled corticosteroids (see section 4.2).

SINGULAIR is also indicated in the prophylaxis of asthma from 2 years of age and older in which the predominant component is exercise-induced bronchoconstriction.

4.2 Posology and method of administration

This medicinal product is to be given to a child under adult supervision. The dosage for paediatric patients 6 months to 5 years of age is one sachet of 4-mg granules daily to be taken in the evening. No dosage adjustment within this age group is necessary. Efficacy data from clinical trials in paediatric patients 6 months to 2 years of age with persistent asthma are limited. Patients should be evaluated after 2 to 4 weeks for response to montelukast treatment. Treatment should be discontinued if a lack of response is observed. The SINGULAIR Paediatric 4 mg granules formulation is not recommended below 6 months of age.

Administration of SINGULAIR granules:

SINGULAIR granules can be administered either directly in the mouth, or mixed with a spoonful of cold or room temperature soft food (e.g., applesauce, ice cream, carrots and rice). The sachet should not be opened until ready to use. After opening the sachet, the full dose of SINGULAIR granules must be administered immediately (within 15 minutes). If mixed with food, SINGULAIR granules must not be stored for future use. SINGULAIR granules are not intended to be dissolved in liquid for administration. However, liquids may be taken subsequent to administration. SINGULAIR granules can be administered without regard to the timing of food ingestion.

General recommendations. The therapeutic effect of SINGULAIR on parameters of asthma control occurs within one day. Patients should be advised to continue taking SINGULAIR even if their asthma is under control, as well as during periods of worsening asthma.

No dosage adjustment is necessary for patients with renal insufficiency, or mild to moderate hepatic impairment. There are no data on patients with severe hepatic impairment. The dosage is the same for both male and female patients.

SINGULAIR as an alternative treatment option to low-dose inhaled corticosteroids for mild, persistent asthma:

Montelukast is not recommended as monotherapy in patients with moderate persistent asthma. The use of montelukast as an alternative treatment option to low-dose inhaled corticosteroids for children 2 to 5 years old with mild persistent asthma should only be considered for patients who do not have a recent history of serious asthma attacks that required oral corticosteroid use and who have demonstrated that

they are not capable of using inhaled corticosteroids (see section 4.1). Mild persistent asthma is defined as asthma symptoms more than once a week but less than once a day, nocturnal symptoms more than twice a month but less than once a week, normal lung function between episodes. If satisfactory control of asthma is not achieved at follow-up (usually within one month), the need for an additional or different anti-inflammatory therapy based on the step system for asthma therapy should be evaluated. Patients should be periodically evaluated for their asthma control.

SINGULAIR as prophylaxis of asthma for 2 to 5 year old patients in whom the predominant component is exercise-induced bronchoconstriction.

In 2 to 5 year old patients, exercise-induced bronchoconstriction may be the predominant manifestation of persistent asthma that requires treatment with inhaled corticosteroids. Patients should be evaluated after 2 to 4 weeks of treatment with montelukast. If satisfactory response is not achieved, an additional or different therapy should be considered.

Therapy with SINGULAIR in relation to other treatments for asthma.

When treatment with SINGULAIR is used as add-on therapy to inhaled corticosteroids, SINGULAIR should not be abruptly substituted for inhaled corticosteroids (see section 4.4).

10-mg film-coated tablets are available for adults 15 years of age and older.

5-mg chewable tablets are available for paediatric patients 6 to 14 years of age.

4-mg chewable tablets are available as an alternative formulation for paediatric patients 2 to 5 years of age.

4.3 Contraindications

Hypersensitivity to the active substance or to any of the excipients.

4.4 Special warnings and precautions for use

The diagnosis of persistent asthma in very young children (6 months – 2 years) should be established by a paediatrician or pulmonologist.

Patients should be advised never to use oral montelukast to treat acute asthma attacks and to keep their usual appropriate rescue medication for this purpose readily available. If an acute attack occurs, a short-acting inhaled β_2 agonist should be used. Patients should seek their doctors' advice as soon as possible if they need more inhalations of short-acting β_2 -agonists than usual.

Montelukast should not be abruptly substituted for inhaled or oral corticosteroids.

There are no data demonstrating that oral corticosteroids can be reduced when montelukast is given concomitantly.

In rare cases, patients on therapy with anti-asthma agents including montelukast may present with systemic eosinophilia, sometimes presenting with clinical features of vasculitis consistent with Churg-Strauss syndrome, a condition which is often treated with systemic corticosteroid therapy. These cases usually, but not always, have been associated with the reduction or withdrawal of oral corticosteroid therapy. The possibility that leukotriene receptor antagonists may be associated with emergence of

Churg-Strauss syndrome can neither be excluded nor established. Physicians should be alert to eosinophilia, vasculitic rash, worsening pulmonary symptoms, cardiac complications, and/or neuropathy presenting in their patients. Patients who develop these symptoms should be reassessed and their treatment regimens evaluated.

4.5 Interaction with other medicinal products and other forms of interaction

Montelukast may be administered with other therapies routinely used in the prophylaxis and chronic treatment of asthma. In drug-interactions studies, the recommended clinical dose of montelukast did not have clinically important effects on the pharmacokinetics of the following medicinal products : theophylline, prednisone, prednisolone, oral contraceptives (ethinyl estradiol/norethindrone 35/1), terfenadine, digoxin and warfarin.

The area under the plasma concentration curve (AUC) for montelukast was decreased approximately 40% in subjects with co-administration of phenobarbital. Since montelukast is metabolised by CYP 3A4, caution should be exercised, particularly in children, when montelukast is co-administered with inducers of CYP 3A4, such as phenytoin, phenobarbital and rifampicin.

In vitro studies have shown that montelukast is a potent inhibitor of CYP 2C8. However, data from a clinical drug – drug interaction study involving montelukast and rosiglitazone (a probe substrate representative of medicinal products primarily metabolised by CYP 2C8) demonstrated that montelukast does not inhibit CYP 2C8 *in vivo*. Therefore, montelukast is not anticipated to markedly alter the metabolism of medicinal products metabolised by this enzyme (e.g., paclitaxel, rosiglitazone, and repaglinide.)

4.6 Pregnancy and lactation

Use during pregnancy

Animal studies do not indicate harmful effects with respect to effects on pregnancy or embryonal/foetal development.

Limited data from available pregnancy databases do not suggest a causal relationship between SINGULAIR and malformations (i.e. limb defects) that have been rarely reported in worldwide post marketing experience.

SINGULAIR may be used during pregnancy only if it is considered to be clearly essential.

Use during lactation

Studies in rats have shown that montelukast is excreted in milk (see section 5.3). It is not known if montelukast is excreted in human milk.

SINGULAIR may be used in breast-feeding mothers only if it is considered to be clearly essential.

4.7 Effects on ability to drive and use machines

Montelukast is not expected to affect a patient's ability to drive a car or operate machinery. However, in very rare cases, individuals have reported drowsiness or dizziness.

4.8 Undesirable effects

Montelukast has been evaluated in clinical studies in patients with persistent asthma as follows:

- 10-mg film-coated tablets in approximately 4000 adult patients 15 years of age and older
- 5-mg chewable tablets in approximately 1750 paediatric patients 6 to 14 years of age
- 4-mg chewable tablets in 851 paediatric patients 2 to 5 years of age, and
- 4-mg granules in 175 paediatric patients 6 months to 2 years of age.

Montelukast has been evaluated in a clinical study in patients with intermittent asthma as follows:

- 4 mg granules and chewable tablets in 1038 paediatric patients 6 months to 5 years of age

The following drug-related adverse reactions in clinical studies were reported commonly ($\geq 1/100$ to $<1/10$) in patients treated with montelukast and at a greater incidence than in patients treated with placebo:

Body System Class	Adult Patients 15 years and older (two 12-week studies; n=795)	Paediatric Patients 6 to 14 years old (one 8-week study; n=201) (two 56-week studies; n=615)	Paediatric Patients 2 to 5 years old (one 12-week study; n=461) (one 48-week study; n=278)	Paediatric Patients 6 months up to 2 years old (one 6-week study; n=175)
Nervous system disorders	headache	headache		hyperkinesia
Respiratory, thoracic, and mediastinal disorders				asthma
Gastro-intestinal disorders	abdominal pain		abdominal pain	diarrhoea
Skin and subcutaneous tissue disorders				eczematous dermatitis, rash
General disorders and administration site conditions			thirst	

With prolonged treatment in clinical trials with a limited number of patients for up to 2 years for adults, and up to 12 months for paediatric patients 6 to 14 years of age, the safety profile did not change.

Cumulatively, 502 paediatric patients 2 to 5 years of age were treated with montelukast for at least 3 months,

338 for 6 months or longer, and 534 patients for 12 months or longer. With prolonged treatment, the safety profile did not change in these patients either.

The safety profile in paediatric patients 6 months to 2 years of age did not change with treatment up to 3 months.

The following adverse reactions have been reported in post-marketing use:

Infections and infestations: upper respiratory infection.

Blood and lymphatic system disorders: increased bleeding tendency.

Immune system disorders: hypersensitivity reactions including anaphylaxis, hepatic eosinophilic infiltration.

Psychiatric disorders: dream abnormalities including nightmares, hallucinations, insomnia, somnambulism, irritability, anxiety, restlessness, agitation including aggressive behaviour or hostility, tremor, depression, suicidal thinking and behaviour (suicidality) in very rare cases.

Nervous system disorders: dizziness, drowsiness, paraesthesia/hypoesthesia, seizure.

Cardiac disorders: palpitations.

Respiratory, thoracic and mediastinal disorders: epistaxis.

Gastro-intestinal disorders: diarrhoea, dry mouth, dyspepsia, nausea, vomiting.

Hepatobiliary disorders: elevated levels of serum transaminases (ALT, AST), hepatitis (including cholestatic, hepatocellular and mixed pattern liver injury).

Skin and subcutaneous tissue disorders: angioedema, bruising, urticaria, pruritus, rash, erythema nodosum.

Musculoskeletal and connective tissue disorders: arthralgia, myalgia including muscle cramps.

General disorders and administration site conditions: asthenia/fatigue, malaise, oedema, pyrexia.

Very rare cases of Churg-Strauss Syndrome (CSS) have been reported during montelukast treatment in asthmatic patients (see section 4.4).

4.9 Overdose

No specific information is available on the treatment of overdose with montelukast. In chronic asthma studies, montelukast has been administered at doses up to 200 mg/day to adult patients for 22 weeks and in short term studies, up to 900 mg/day to patients for approximately one week without clinically important adverse experiences.

There have been reports of acute overdose in post-marketing experience and clinical studies with montelukast. These include reports in adults and children with a dose as high as 1000 mg (approximately 61 mg/kg in a 42 month old child). The clinical and laboratory findings observed were consistent with the safety profile in adults and paediatric patients. There were no adverse experiences in the majority of overdose reports. The most frequently occurring adverse experiences were consistent with the safety profile of montelukast and included abdominal pain, somnolence, thirst, headache, vomiting, and psychomotor hyperactivity.

It is not known whether montelukast is dialysable by peritoneal- or haemo-dialysis.

5. PHARMACOLOGICAL PROPERTIES

5.1 Pharmacodynamic properties

Pharmacotherapeutic group: Leukotriene receptor antagonist

ATC-code: R03D C03

The cysteinyl leukotrienes (LTC₄, LTD₄, LTE₄) are potent inflammatory eicosanoids released from various cells including mast cells and eosinophils. These important pro-asthmatic 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. In clinical studies, montelukast inhibits bronchoconstriction due to inhaled LTD₄ at doses as low as 5 mg. Bronchodilation was observed within 2 hours of oral administration. The bronchodilation effect caused by a β^- agonist was additive to that caused by montelukast. Treatment with montelukast inhibited both early- and late-phase bronchoconstriction due to antigen challenge. Montelukast, compared with placebo, decreased peripheral blood eosinophils in adult and paediatric patients. In a separate study, treatment with montelukast significantly decreased eosinophils in the airways (as measured in sputum). In adult and paediatric patients 2 to 14 years of age, montelukast, compared with placebo, decreased peripheral blood eosinophils while improving clinical asthma control.

In studies in adults, montelukast, 10 mg once daily, compared with placebo, demonstrated significant improvements in morning FEV₁ (10.4% vs 2.7% change from baseline), AM peak expiratory flow rate (PEFR) (24.5 L/min vs 3.3 L/min change from baseline), and significant decrease in total β^- agonist use (-26.1% vs -4.6% change from baseline). Improvement in patient-reported daytime and night-time asthma symptoms scores was significantly better than placebo.

Studies in adults demonstrated the ability of montelukast to add to the clinical effect of inhaled corticosteroid (% change from baseline for inhaled beclometasone plus montelukast vs beclometasone, respectively for FEV₁: 5.43% vs 1.04%; β^- agonist use: -8.70% vs 2.64%). Compared with inhaled beclometasone (200 μ g twice daily with a spacer device), montelukast demonstrated a more rapid initial response, although over the 12-week study, beclometasone provided a greater average treatment effect (% change from baseline for montelukast vs beclometasone, respectively for FEV₁: 7.49% vs 13.3%; β^- agonist use: -28.28% vs -43.89%). However, compared with beclometasone, a high percentage of patients treated with montelukast achieved similar clinical responses (e.g., 50% of patients treated with beclometasone achieved an improvement in FEV₁ of approximately 11% or more over baseline while approximately 42% of patients treated with montelukast achieved the same response).

In an 8-week study in paediatric patients 6 to 14 years of age, montelukast 5 mg once daily, compared with placebo, significantly improved respiratory function (FEV₁ 8.71% vs 4.16% change from baseline; AM PEFR 27.9 L/min vs 17.8 L/min change from baseline) and decreased "as-needed" β^- agonist use (-11.7% vs +8.2% change from baseline).

In a 12-month study comparing the efficacy of montelukast to inhaled fluticasone on asthma control in paediatric patients 6 to 14 years of age with mild persistent asthma, montelukast was non-inferior to fluticasone in increasing the percentage of asthma rescue-free days (RFDs), the primary endpoint. Averaged over the 12-month treatment period, the percentage of asthma RFDs increased from 61.6 to

84.0 in the montelukast group and from 60.9 to 86.7 in the fluticasone group. The between group difference in LS mean increase in the percentage of asthma RFDs was statistically significant (-2.8 with a 95% CI of -4.7, -0.9), but within the limit pre-defined to be clinically not inferior. Both montelukast and fluticasone also improved asthma control on secondary variables assessed over the 12 month treatment period:

- FEV₁ increased from 1.83 L to 2.09 L in the montelukast group and from 1.85 L to 2.14 L in the fluticasone group. The between-group difference in LS mean increase in FEV₁ was -0.02 L with a 95% CI of -0.06, 0.02. The mean increase from baseline in % predicted FEV₁ was 0.6% in the montelukast treatment group, and 2.7% in the fluticasone treatment group. The difference in LS means for the change from baseline in the % predicted FEV₁ was significant: -2.2% with a 95% CI of -3.6, -0.7.
- The percentage of days with β -agonist use decreased from 38.0 to 15.4 in the montelukast group, and from 38.5 to 12.8 in the fluticasone group. The between group difference in LS means for the percentage of days with β -agonist use was significant: 2.7 with a 95% CI of 0.9, 4.5.
- The percentage of patients with an asthma attack (an asthma attack being defined as a period of worsening asthma that required treatment with oral steroids, an unscheduled visit to the doctor's office, an emergency room visit, or hospitalisation) was 32.2 in the montelukast group and 25.6 in the fluticasone group; the odds ratio (95% CI) being significant: equal to 1.38 (1.04, 1.84).
- The percentage of patients with systemic (mainly oral) corticosteroid use during the study period was 17.8% in the montelukast group and 10.5% in the fluticasone group. The between group difference in LS means was significant: 7.3% with a 95% CI of 2.9; 11.7.

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 -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.

In a placebo-controlled study in paediatric patients 6 months to 5 years of age who had intermittent asthma but did not have persistent asthma, treatment with montelukast was administered over a 12-month period, either as a once-daily 4 mg regimen or as a series of 12-day courses that each were started when an episode of intermittent symptoms began. No significant difference was observed between patients treated with montelukast 4 mg or placebo in the number of asthma episodes culminating in an asthma attack, defined as an asthma episode requiring utilization of health-care resources such as an unscheduled visit to a doctor's office, emergency room, or hospital; or treatment with oral, intravenous, or intramuscular corticosteroid.

Efficacy of montelukast is supported in paediatric patients 6 months to 2 years of age by extrapolation from the demonstrated efficacy in patients 2 years of age and older with asthma, and is based on similar pharmacokinetic data, as well as the assumption that the disease course, pathophysiology and the

medicinal products's effect are substantially similar among these populations.

Significant reduction of exercise-induced bronchoconstriction (EIB) was demonstrated in a 12-week study in adults (maximal fall in FEV₁ 22.33% for montelukast vs 32.40% for placebo; time to recovery to within 5% of baseline FEV₁ 44.22 min vs 60.64 min). This effect was consistent throughout the 12-week study period. Reduction in EIB was also demonstrated in a short term study in paediatric patients 6 to 14 years of age (maximal fall in FEV₁ 18.27% vs 26.11%; time to recovery to within 5% of baseline FEV₁ 17.76 min vs 27.98 min). The effect in both studies was demonstrated at the end of the once-daily dosing interval.

In aspirin-sensitive asthmatic patients receiving concomitant inhaled and/or oral corticosteroids, treatment with montelukast, compared with placebo, resulted in significant improvement in asthma control (FEV₁ 8.55% vs -1.74% change from baseline and decrease in total β₂-agonist use -27.78% vs 2.09% change from baseline).

5.2 Pharmacokinetic properties

Absorption. Montelukast is rapidly absorbed following oral administration. For the 10-mg film-coated tablet, the mean peak plasma concentration (C_{max}) is achieved 3 hours (T_{max}) after administration in adults in the fasted state. The mean oral bioavailability is 64%. The oral bioavailability and C_{max} are not influenced by a standard meal. Safety and efficacy were demonstrated in clinical trials where the 10-mg film-coated tablet was administered without regard to the timing of food ingestion.

For the 5-mg chewable tablet, the C_{max} is achieved in 2 hours after administration in adults in the fasted state. The mean oral bioavailability is 73% and is decreased to 63% by a standard meal.

After administration of the 4-mg chewable tablet to paediatric patients 2 to 5 years of age in the fasted state, C_{max} is achieved 2 hours after administration. The mean C_{max} is 66% higher while mean C_{min} is lower than in adults receiving a 10-mg tablet.

The 4-mg granule formulation is bioequivalent to the 4-mg chewable tablet when administered to adults in the fasted state. In paediatric patients 6 months to 2 years of age, C_{max} is achieved 2 hours after administration of the 4-mg granules formulation. C_{max} is nearly 2-fold greater than in adults receiving a 10-mg tablet. The co-administration of applesauce or a high-fat standard meal with the granule formulation did not have a clinically meaningful effect on the pharmacokinetics of montelukast as determined by AUC (1225.7 vs 1223.1 ng·hr/mL with and without applesauce, respectively, and 1191.8 vs 1148.5 ng·hr/mL with and without a high-fat standard meal, respectively).

Distribution. Montelukast is more than 99% bound to plasma proteins. The steady-state volume of distribution of montelukast averages 8-11 litres. Studies in rats with radiolabelled montelukast indicate minimal distribution across the blood-brain barrier. In addition, concentrations of radiolabelled material at 24 hours post-dose were minimal in all other tissues.

Biotransformation. Montelukast is extensively metabolised. In studies with therapeutic doses, plasma concentrations of metabolites of montelukast are undetectable at steady state in adults and children.

In vitro studies using human liver microsomes indicate that cytochrome P450 3A4, 2A6 and 2C9 are involved in the metabolism of montelukast. Based on further *in vitro* results in human liver microsomes, therapeutic plasma concentrations of montelukast do not inhibit cytochromes P450 3A4, 2C9, 1A2, 2A6, 2C19, or 2D6. The contribution of metabolites to the therapeutic effect of montelukast is minimal.

Elimination. The plasma clearance of montelukast averages 45 ml/min in healthy adults. Following an

oral dose of radiolabelled montelukast, 86% of the radioactivity was recovered in 5-day faecal collections and <0.2% was recovered in urine. Coupled with estimates of montelukast oral bioavailability, this indicates that montelukast and its metabolites are excreted almost exclusively via the bile.

Characteristics in patients. No dosage adjustment is necessary for the elderly or mild to moderate hepatic insufficiency. Studies in patients with renal impairment have not been undertaken. Because montelukast and its metabolites are eliminated by the biliary route, no dose adjustment is anticipated to be necessary in patients with renal impairment. There are no data on the pharmacokinetics of montelukast in patients with severe hepatic insufficiency (Child-Pugh score >9).

With high doses of montelukast (20- and 60-fold the recommended adult dose), a decrease in plasma theophylline concentration was observed. This effect was not seen at the recommended dose of 10 mg once daily.

5.3 Preclinical safety data

In animal toxicity studies, minor serum biochemical alterations in ALT, glucose, phosphorus and triglycerides were observed which were transient in nature. The signs of toxicity in animals were increased excretion of saliva, gastro-intestinal symptoms, loose stools and ion imbalance. These occurred at dosages which provided >17-fold the systemic exposure seen at the clinical dosage. In monkeys, the adverse effects appeared at doses from 150 mg/kg/day (>232-fold the systemic exposure seen at the clinical dose). In animal studies, montelukast did not affect fertility or reproductive performance at systemic exposure exceeding the clinical systemic exposure by greater than 24-fold. A slight decrease in pup body weight was noted in the female fertility study in rats at 200 mg/kg/day (>69-fold the clinical systemic exposure). In studies in rabbits, a higher incidence of incomplete ossification, compared with concurrent control animals, was seen at systemic exposure >24-fold the clinical systemic exposure seen at the clinical dose. No abnormalities were seen in rats. Montelukast has been shown to cross the placental barrier and is excreted in breast milk of animals.

No deaths occurred following a single oral administration of montelukast sodium at doses up to 5000 mg/kg in mice and rats (15,000 mg/m² and 30,000 mg/m² in mice and rats, respectively), the maximum dose tested. This dose is equivalent to 25,000 times the recommended daily adult human dose (based on an adult patient weight of 50 kg).

Montelukast was determined not to be phototoxic in mice for UVA, UVB or visible light spectra at doses up to 500 mg/kg/day (approximately >200-fold based on systemic exposure).

Montelukast was neither mutagenic in *in vitro* and *in vivo* tests nor tumorigenic in rodent species.

6. PHARMACEUTICAL PARTICULARS

6.1 List of excipients

Mannitol

Hypolose (E 463)

Magnesium stearate

6.2 Incompatibilities

Not applicable.

6.3 Shelf life

2 years.

6.4 Special precautions for storage

Store in the original package in order to protect from light and moisture.

6.5 Nature and contents of container

Packaged in polyethylene/aluminum/polyester sachet in:

Cartons of 7, 20, 28 and 30 sachets.

Not all pack sizes may be marketed.

6.6 Special precautions for disposal and other handling

Any unused product or waste material should be disposed of in accordance with local requirements.

7. MARKETING AUTHORISATION HOLDER

Merck Sharp & Dohme Limited

Hertford Road, Hoddesdon, Hertfordshire EN11 9BU, UK

8. MARKETING AUTHORISATION NUMBER(S)

PL 0025/0440

9. DATE OF FIRST AUTHORISATION/RENEWAL OF THE AUTHORISATION

14 February 2003/ 25 August 2007

10. DATE OF REVISION OF THE TEXT

November 2010

LEGAL CATEGORY

POM

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SPC.SGA-OG.10.UK.3247.II-052

Placebo certificate of analysis

Nova Laboratories Limited**Certificate Of Analysis**

Page 1 of 1

Product:	Placebo to match Montelukast 4mg Paediatric Granules
Batch Number:	0891x001

RELEASE TESTS

Test	Method Reference	Acceptance criteria	Result	Pass / Fail
Appearance	CM0891	White Granules	White Granules	Pass
Absence of Montelukast by HPLC	CM0891	No response at the retention time for Montelukast	No response at the retention time for Montelukast	Pass
Uniformity of Mass (Ph Eur 2.9.5)	CM0891	Complies with Ph Eur	Conforms	Pass
Total Viable Count	SOP1034	TAMC: NMT 2000 cfu/g TYMC: NMT 200 cfu/g	< 4 cfu / g < 4 cfu / g	Pass

[Redacted Signature]

Julie Walker
Head of Quality

[Redacted Signature]

Audrey Holt
Quality Systems Manager

Date: 08 Sep 10

Date: 08 Sep 10

Audit certificate (Medicines and Healthcare Products Regulatory Agency Good Medical Practice Inspection – Novalabs)

GMP INSPECTION OF Nova Laboratories Ltd Martin House, Gloucester Crescent, Wigston Leicester LE18 4YL	MHRA GMP 13581/4097	PAGE 2 of 18
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GMP INSPECTION OF Nova Laboratories Ltd

SECTION A INSPECTION REPORT SUMMARY

Inspection requested by: Routine fee based re-inspection

Scope of Inspection: EU Guide to GMP

Licence or Reference Number: MIA, MIA(IMP), MS and ManSA 13581

Licence Holder/Applicant: Nova Laboratories Ltd

Details of Product(s)/ Clinical trials/Studies:
Variety of aseptically prepared sterile products, for Clinical Trials or hospital specials.
Extemporaneously prepared non-sterile products as specials.

Activities carried out by company:

Manufacture of Active Ingredients	Y/N
Manufacture of Finished Medicinal Products	N
Manufacture of Intermediate or Bulk	Y
Packaging	Y
Importing	Y
Laboratory Testing	N
Batch Certification and Batch Release	Y
Other: Specials and IMP activities	Y

Name and Address of site(s) inspected: Nova Laboratories Ltd
Martin House, Gloucester Crescent,
Wigston Leicester LE18 4YL

Site Contact: Dr Peter White peter.white@novalabs.co.uk

Date(s) of Inspection: 11-15th July 2011

Lead Inspector: Vicki Pike

Accompanying Inspector(s): N/A

References: Insp GMP/IMP 13581/4097-0015

Final Conclusion/Recommendation:

The site operates to a satisfactory level of GMP. A GMP certificate shall be issued. Please refer to Annex 1 for re-inspection frequency.

Name and Dated Signature of Lead Inspector:

Signed:

Dated: 16/08/11

Safeguarding public health		<h2>INSPECTION REPORT</h2> <p>Nova Laboratories Ltd Martin House Gloucester Crescent Wigston Leicester LE18 4YL</p>	<p>Medicines and Healthcare Products Regulatory Agency 2A Hunter House 57 Goodramgate YORK YO1 7FX Telephone: 01904 610556 Fax: 01904 625430</p>	<p>Head Office: Inspection & Standards Division, Medicines and Healthcare products Regulatory Agency 151 Buckingham Palace Road, Victoria, London SW1W 9SZ Tel: 020 3080 6000</p>	<p>Medicines and Healthcare products Regulatory Agency</p>

Appendix 9 CONSORT checklist

CONSORT 2010 checklist of information to include when reporting a randomised trial*			
Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract	1a	Identification as a randomised trial in the title	i
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	vii, viii
Introduction	2a	Scientific background and explanation of rationale	1–2
	2b	Specific objectives or hypotheses	2
Methods	3a	Description of trial design (such as parallel, factorial) including allocation ratio	3–7
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5, 97
Participants	4a	Eligibility criteria for participants	4
	4b	Settings and locations where the data were collected	4
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	8–10
	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	10–12
Outcomes	6b	Any changes to trial outcomes after the trial commenced, with reasons	28
	7a	How sample size was determined	12
Sample size	7b	When applicable, explanation of any interim analyses and stopping guidelines	N/A
	8a	Method used to generate the random allocation sequence	5
Randomisation:	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5–7
	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	5–7
Allocation concealment mechanism	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5–7
	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	7
Blinding	11b	If relevant, description of the similarity of interventions	8

Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	12, 75
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	12, 75
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	15–17
Recruitment	13b	For each group, losses and exclusions after randomisation, together with reasons	15–17
	14a	Dates defining the periods of recruitment and follow-up	13
	14b	Why the trial ended or was stopped	13
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	18
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	16, 17
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	19–21
Ancillary analyses	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	N/A
	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	19–21
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	23–25
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	29
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	29
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	29
Other information			
Registration	23	Registration number and name of trial registry	viii
Protocol	24	Where the full trial protocol can be accessed, if available	www.nets.nih.gov/uc/uk_data/assets/pdf/file/0004/52942/PRO-08-43-03.pdf (not stated in manuscript)
Funding	25	Sources of funding and other support (such as supply of drugs), role of funder	viii

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org

Appendix 10 CONSORT checklist for abstracts

Item	Description	Reported on line number
Title	Identification of the study as randomised	14
Authors	Contact details for the corresponding author	c.nwokoro@qmul.ac.uk
Trial design	Description of the trial design (e.g. parallel, cluster, non-inferiority)	14
Methods		
Participants	Eligibility criteria for participants and the settings where the data were collected	19
Interventions	Interventions intended for each group	24
Objective	Specific objective or hypothesis	9
Outcome	Clearly defined primary outcome for this report	29
Randomisation	How participants were allocated to interventions	31
Blinding (masking)	Whether or not participants, care givers, and those assessing the outcomes were blinded to group assignment	34
Results		
Numbers randomised	Number of participants randomised to each group	42
Recruitment	Trial status	42
Numbers analysed	Number of participants analysed in each group	43
Outcome	For the primary outcome, a result for each group and the estimated effect size and its precision	45
Harms	Important adverse events or side effects	50
Conclusions	General interpretation of the results	52
Trial registration	Registration number and name of trial register	66
Funding	Source of funding	65

A decorative graphic consisting of numerous thin, parallel green lines that curve from the left side of the page towards the right, creating a sense of movement and depth.

**EME
HS&DR
HTA
PGfAR
PHR**

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