The Azithromycin for Acute Exacerbations of Asthma (AZALEA) Trial

This supplement contains the following items:

1. Original protocol (pages 2 to 64), final protocol (pages 65 to 116) and summary of changes (page 117)

2. Original statistical analysis plan (pages 118 to 154) and summary of changes between original plan and final analyses undertaken (155 to 156) – please note that no changes were made to the SAP after the original version so this is also the final version and we have given a note of changes between the final version and the analyses that was undertaken. Please also note that the final version of the SAP states ‘draft’ in error and this is the final version on which analysis was based.
CLINICAL STUDY PROTOCOL

AZALEA Study

A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma

Version 1, 18th April 2011

REC reference: *****TBC*****

EudraCT reference: 2011-001093-26

Protocol Authorised by:  Date  Signature


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PROTOCOL OUTLINE

Full Title
A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma

Short Title/Acronym
AZALEA
AZithromycin Against pLacebo in Exacerbations of Asthma

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Sponsor
Imperial College Academic Health Science Centre is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:
Objective
To evaluate the efficacy of azithromycin during an acute exacerbation of asthma.

Design
Multi-centre, randomised, double-blind, placebo-controlled study

Population
Adults with a history of asthma presenting within 24 hours (of initial presentation requesting medical care) with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough with reduced PEF)

Sample size
Approximately 380 patients will be enrolled, with the goal of obtaining approximately 190 clinically and microbiologically evaluable patients per treatment arm.

Efficacy Assessment Principal Criteria

Primary Outcome
Diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

Secondary Outcomes
- The following additional efficacy endpoints:
  - Health status assessed by acute asthma QoLQ (Juniper)
  - Health status assessed by Mini Asthma QoLQ (Juniper)
  - Pulmonary Function tests (FEV1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes will be assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies (the efficacy of Telithromycin was only assessed at 10 days).
- Time to 50% reduction in symptom score
**Exploratory analyses**
- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial *C. pneumoniae* and/or *M. pneumoniae* status
- Assessment of efficacy outcomes in relation to initial standard bacteriologic status
- Assessment of efficacy outcomes in relation to initial virologic status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

**Clinical Safety Assessment**
Adverse event reporting, physical examinations, vital signs, and clinical laboratory parameters.

Additionally, a hierarchical model at 3 levels (including day at the lowest level) will be fitted to allow an assessment of trends over time in primary and secondary endpoints.

Analysis will be performed on an intention to treat (ITT) basis.

**Study duration and dates**
The study will start June 1st 2011 for development of CRFs and training of personnel. Subject recruitment is proposed to last 1 year to start 1st September 2011 and end in August 2012. Laboratory analyses, data cleaning, analysis and reporting will take a further 9 months.
## STUDY SCHEDULE

### Flow Chart of Study Procedures

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Visit 1 Day 1 Within 24 hrs of initial presentation</th>
<th>Visit 2 Day 5</th>
<th>Visit 3 Day 10</th>
<th>Visit 4 Follow up Visit Day 42</th>
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<tr>
<td>Informed consent</td>
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<td>Inclusion/Exclusion criteria review</td>
<td>X</td>
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<tr>
<td>Demographics</td>
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<td>Medical/Surgical history</td>
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<tr>
<td>Record previous &amp; concomitant treatments</td>
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<tr>
<td>Pulmonary function tests (fev₁, fvc, fev₁/fvc ratio, fef₂₅-₇₅%, fef ₅₀% peak flow)</td>
<td>X X X</td>
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<tr>
<td>Urine pregnancy test*</td>
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<td>Serology for atypical pathogens</td>
<td>X</td>
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<tr>
<td>Nasal swab/aspirate for PCR</td>
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<tr>
<td>Spontaneous/induced sputum for PCR</td>
<td>X</td>
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<tr>
<td>Culture of sputum for standard bacteria (quantitative)</td>
<td>X</td>
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<tr>
<td>Sputum for cell differential and mediators in supernatant</td>
<td>X</td>
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<tr>
<td>Full Blood Count (FBC)</td>
<td>X</td>
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<tr>
<td>Dispense diary- Diary training</td>
<td>X</td>
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<tr>
<td>Diary review</td>
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<tr>
<td>Return Diary to investigator</td>
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<tr>
<td>Health outcomes assessment - Acute Asthma QoLQ (Juniper)</td>
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<tr>
<td>Health outcomes assessment - MiniAQLQ (Juniper)</td>
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<tr>
<td>Randomisation and Dispense study medication</td>
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<td>Collect and count unused drug</td>
<td>X</td>
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<tr>
<td>AE review</td>
<td>X X X X</td>
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* if indicated
# ABBREVIATIONS AND DEFINITIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AR</td>
<td>Adverse reaction</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>FEF&lt;sub&gt;25-75%&lt;/sub&gt;</td>
<td>Forced Mid-Expiratory Flow Rate</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC</td>
<td>Ratio of forced expiratory volume in one second to forced vital capacity</td>
</tr>
<tr>
<td>GCP</td>
<td>Good clinical practice</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroids</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-to-treat</td>
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<tr>
<td>MHRA</td>
<td>Medicines and Healthcare Products Regulatory Agency</td>
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<tr>
<td>OCS</td>
<td>Oral corticosteroids</td>
</tr>
<tr>
<td>PC</td>
<td>Predefined change</td>
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<tr>
<td>PCA</td>
<td>Predefined change abnormal</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function tests</td>
</tr>
<tr>
<td>REC</td>
<td>Research ethics committee</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SAR</td>
<td>Serious adverse reaction</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
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</table>
1. INTRODUCTION AND STUDY RATIONALE

Importance of asthma exacerbations
Asthma is the most prevalent respiratory disease, it is diagnosed in 5-10% of adults and 10-15% of children and around 30% of children report wheeze in the last year. The major asthma morbidity and mortality are a result of acute exacerbations: 5-10% of asthmatics have been hospitalised with an exacerbation and ~25,000 Europeans die unnecessarily of asthma each year. Exacerbations also account for ~50% of total expenditure on asthma care. More than half of asthma patients reported having an exacerbation in the last year with >1/3 children and >1/4 adults requiring urgent medical care visits as a result.

Aetiology of asthma exacerbations

Viruses and atypical bacteria: Respiratory viral infections are the major cause of asthma exacerbations in children (80-85%) and adults (75-80%). However, non-viral respiratory pathogens such as Mycoplasma pneumoniae (M. pneumoniae) and Chlamydia pneumoniae (C. pneumoniae) have also been associated with wheezing episodes and asthma exacerbations in both adults and children. Interestingly, in two of these studies virus detection rates were ~80%, while serologic positivity for atypical bacterial infection/reactivation can be as high as 40-60%, indicating that viral and atypical bacterial infections likely interact in increasing risk of asthma exacerbation.

Bacterial infections: There is little published evidence that standard bacterial infections are important in the aetiology of asthma exacerbations, however, patients with asthma have increased susceptibility to respiratory bacterial infections, increased carriage of pathogenic respiratory bacteria identified by culture and molecular techniques and impaired interferon responses to bacterial polysaccharides. In addition viral infection impairs innate responses important in antibacterial immunity and increases bacterial adherence to bronchial epithelial cells. There is therefore good evidence that bacterial respiratory infections are both more common and more severe in asthma, and that viral infection can increase susceptibility to bacterial infection.

A recent study of 361 children with >800 stable and exacerbation airway samples collected during the first 3 years of life and analysed for standard bacteria and respiratory viruses, has confirmed that acute wheezing episodes were associated with both bacterial infection (odds-ratio 2.9, 95% CI 1.9-4.3, p<0.001) and with virus infection (odds-ratio 2.8, 95% CI [1.7, 4.4], p<0.01). We therefore hypothesise that standard bacterial infections are likely also to be important in the aetiology of asthma exacerbations in adults, and aim to investigate this in the proposed study.

Treatment of asthma exacerbations
When asthma exacerbations occur, treatment options are limited to bronchodilators and steroids. Beyond the addition of magnesium in severe exacerbations, treatments have developed very little in the last ~50 years. Current therapeutic strategies are of limited efficacy and development of new approaches addressing the aetiologic agents causing the exacerbations is urgently needed. Current asthma guidelines recommend specifically that antibiotic therapy should NOT be administered routinely in asthma exacerbations.

New approaches to treatment of asthma exacerbations
If atypical bacteria are causal or contributory factors in asthma exacerbation, then treatment with antibiotics with activity against mycoplasma and chlamydia species would be expected to be beneficial in asthma exacerbations. Adults with acute exacerbations of asthma and treated with Telithromycin (a ketolide antibiotic closely related to macrolides: both classes being highly active against M. and C. pneumoniae) as a supplement to standard care, showed a statistically significantly greater reduction in asthma symptoms (P<0.005), improvement in lung function (P=0.001) and faster recovery (P=0.03) when compared to those treated with placebo. The magnitude of the treatment effect was also highly clinically significant, with the improvement in symptoms resulting from Telithromycin treatment being ~50% greater than that observed with standard therapy (plus placebo), improvement in lung function being almost 100% greater, and importantly, recovery time to a 50%
improvement in clinical symptoms 3 days faster in those receiving active treatment. This treatment therefore had a clear therapeutic effect; however this study requires confirmation in a second similar study, before revision of guidelines could be considered. Ideally confirmation would be with a further study with Telithromycin, however issues with toxicity have limited use of Telithromycin to severe life threatening infections.

The macrolide antibiotic Azithromycin is a safe and well tolerated alternative that has been used for many years in the treatment of respiratory disease, but has thus far not been studied in acute exacerbations of asthma. We therefore hypothesise that treatment with Azithromycin might be of benefit in treatment of acute asthma exacerbations. This current study will therefore investigate the effectiveness of Azithromycin as a supplement to standard care for adult patients with acute exacerbations of asthma, following as closely as possible the design of the Telithromycin study, with the aim of providing confirmation or otherwise of those results.

**Need for this study**

There are no systematic reviews of, and no published reports of clinical trials investigating efficacy of Azithromycin in the treatment of asthma exacerbations. There are no similar studies registered on Clinicaltrials.gov. The only somewhat similar study is NCT00266851 which plans to enrol 200 adult patients with asthma, either stable persistent or in exacerbation and treat for 3 months, to answer the question: will a 12-week treatment with the antibiotic, Azithromycin, result in a statistically significant and clinically meaningful improvement in overall asthma symptoms and other patient-oriented asthma outcomes one year after initiation of treatment of adult primary care patients with asthma? Thus the aims, design, timing of outcome analysis and treatment length are clearly very different from the proposed study.

**Mechanisms of activity of macrolide/ketolide antibiotics in treatment of asthma exacerbations**

Macrolide/ketolide antibiotics might have therapeutic benefit in treating asthma exacerbations through treatment of either standard or atypical bacteria or both. In addition, both macrolide and ketolide antibiotics have anti-inflammatory properties that are independent of their antibacterial activity which may be beneficial in reducing airway inflammation, which is known to be important in the pathogenesis of asthma exacerbations\(^7,25\). In addition to these three possible mechanisms of action, we also believe antiviral activity is a 4\(^{th}\) possible mechanism.

We have previously reported that impaired type I and type III interferon production by virus infected bronchial epithelial cells and macrophages is important in the pathogenesis of asthma exacerbations\(^20,26\). We have also recently shown that Azithromycin, but not Erythromycin or Telithromycin, significantly increased rhinovirus induced type I and type III interferon and interferon-stimulated anti-viral protein production in primary bronchial epithelial cells, as well as significantly reducing rhinovirus replication and release in bronchial epithelial cells\(^27\). Azithromycin has also been shown to reduce illness severity in a mouse model of viral bronchiolitis\(^28\). Thus Azithromycin has potential to have direct anti-viral activity by augmenting production of those interferons we have already shown to be deficient in asthma exacerbations\(^26,28\), and this property may make it a better treatment option than Telithromycin, which does not appear to have this property\(^27\). A further mechanistic aim of our study therefore, is to investigate frequencies of standard bacterial, atypical bacterial and viral infections in these exacerbations to determine the relative importance of each of these infections, and of possible co-infections with one or more agents, in the aetiology of acute exacerbations of asthma in adult subjects. We will also perform subgroup analyses to determine whether any treatment benefit observed is greater in those with evidence of one or more of these infections, with the aim of shedding some light on the possible mechanism(s) of action of Azithromycin in this context.

**Concerns re antimicrobial resistance**

This clinical trial will be important as there are significant concerns regarding development of resistance against macrolide antibiotics. Although these concerns are somewhat mitigated by the short course of therapy being studied (relative for example to ongoing clinical trials investigating long term treatment in severe asthma), determining whether Azithromycin has efficacy in this context will, if
the study is negative help limit inappropriate use of antibiotics (in a recent study of adult asthma exacerbations, 57% of subjects received antibiotics29).

If the study is positive, determining the frequencies of detection of standard bacterial, atypical bacterial and viral infections in these exacerbations, combined with the subgroup analyses assessing efficacy of the intervention in those with evidence of one or more of these infections should help guide use of such therapies in subgroups of asthma exacerbations that may respond better to such therapies, as well as guiding future investigation of efficacy of alternative antibiotics with shorter durations of action or different spectra of antimicrobial/viral/inflammatory activity.

**Choice of and duration of therapy**

Although the course of therapy is only 3 days, Azithromycin has a multiple-dose tissue half-life of 68hrs and will therefore persist in the lung at significant concentrations for around 10 days after initiation of a 3 day course of therapy30. The main aim of this study is to determine whether the Telithromycin results can be validated in a study with a similar antibiotic, with a similar mechanism and duration of action. Telithromycin was given for 10 days (the standard licensed duration of therapy for other respiratory indications) and the primary outcome variable was assessed at 10 days13. Since our aim is to determine whether the Telithromycin results can be validated, we feel it is important to use the same primary outcome variable and as similar a duration of action as is possible (given that we cannot use Telithromycin due to liver toxicity). This was one reason why we chose to study Azithromycin rather than other macrolide antibiotics. Other reasons for choosing Azithromycin are its antiviral activity that are not shared with other macrolides27, a more favourable drug interaction profile30 and excellent concentration at sites of infection30.

If the study is positive, we will wish to conduct further studies of antibiotics (macrolide and non-macrolide) with shorter durations of action to determine if these are also effective.

**Sputum and serum biomarkers of exacerbation aetiology can be used to direct therapy and improve clinical outcomes in COPD exacerbations**

The Chief Investigator (CI) has recently collaborated with Prof Brightling on a study investigating whether sputum and serum biomarkers of exacerbation aetiology can be used to direct therapy in COPD exacerbations. This study followed patients with COPD for 1 year at stable and exacerbation visits. A large panel of biomarkers (Appendix II) were measured in sputum and blood and viruses, atypical bacteria and standard bacteria were assessed in sputum by PCR and routine diagnostic bacterial culture. Biomarkers that differentiated exacerbations associated with bacteria, viruses or eosinophilic airway inflammation were investigated. 145 patients (101 men, 44 women) entered the study and 182 exacerbations were captured from 86 patients. 55%, 26% and 28% met definitions for bacteria, virus or sputum eosinophil associated exacerbations. Respectively each of the associated exacerbations were best identified by sputum IL-1β (area under receiver operator curve 0.89 (95% confidence interval 0.83 to 0.95), serum CXCL10 (IP-10) 0.83 (0.70 to 0.96), and percentage peripheral blood eosinophils 0.85 (0.78 to 0.93).

In a follow on study, these subjects were randomized to be treated directed by biomarker analysis (patients with blood eosinophilia received steroids, those without did not), or in the non biomarker group all patients received steroid treatment, to determine whether biomarker directed therapy could improve treatment outcomes. In this study all patients received antibiotics. This follow on study found that biomarker directed therapy significantly reduced steroid usage and improved symptom recovery in the biomarker directed group, compared to the non-directed group (Bafadel M, Johnston SL, Brightling CE et al. NEJM [revised and re-submitted]). This study therefore clearly validates the concept that biomarkers of COPD exacerbation phenotypes can be identified and further, that they can be used in directing therapy to improve clinical outcomes in COPD exacerbations.

Serum IP-10 has already been associated with virus induced asthma exacerbations in adults31, however, very few other potential biomarkers have been investigated in this context, and none have been investigated for exacerbations associated with bacterial infections. For this study we propose to collect and store the samples that would permit the biomarker analyses to be carried out at a later date, and if the clinical outcomes of the study are positive, we hope to take forward further projects to
carry out the biomarker analysis. Whether these biomarkers can be applied to direct therapy and improve clinical outcomes will warrant further investigation in future studies in asthma exacerbations.

**Are patterns of airway inflammation associated with aetiology and treatment outcomes?**

Different patterns of airway inflammation have been identified in both stable asthma and during exacerbations – these have been classified as neutrophilic, eosinophilic, mixed granulocytic or pauci-granulocytic. However it is not known whether these different patterns of inflammation are associated with different aetiologies for the exacerbation, nor whether they are related to treatment outcome. We therefore finally aim to characterise the inflammatory cell profiles in sputum at presentation, to determine whether exacerbation aetiology as well as any possible treatment benefit are related to the types of airway inflammation present (neutrophilic, eosinophilic, mixed or pauci-granulocytic).
2. STUDY OBJECTIVES

2.1 Primary Objective

Efficacy will be assessed using diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

2.2 Secondary Objectives

The secondary objectives of the study are to look at:

- The following additional efficacy endpoints:
  - Health status assessed by acute asthma QoLQ (Juniper)
  - Health status assessed by Mini Asthma QoLQ (Juniper)
  - Pulmonary Function tests (FEV1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes will be assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies
- Time to 50% reduction in symptom score

Exploratory analyses

- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial C. pneumoniae and/or M. pneumoniae status
- Assessment of efficacy outcomes in relation to initial standard bacteriologic status
- Assessment of efficacy outcomes in relation to initial virologic status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status
### 3. STUDY DESIGN

This is a multi-centre, randomised, double-blind, placebo-controlled study.

The duration of therapy with study medication (active and/or placebo) will be 3 days, with post-therapy up to 10 days and a follow-up visit at six weeks.

The following diagram summarises the design for the study:
4. SELECTION OF PATIENTS

4.1 Number of patients
As calculated in Section 10.3, page 35, approximately 380 patients should be enrolled and treated with study medication at 10 study centres. The expected enrollment is approximately 38 patients per site. Enrollment into the randomisation phase of the study will be stopped when the anticipated or actual subject numbers have been achieved across all study sites.

4.2 Pre-Randomisation Evaluations
Patients eligible for the study will be approached by a member of the research team within 24 hours of the patient presenting to the hospital with an acute exacerbation of asthma. The patient will be given the study patient information leaflet to read and have the study explained to them. They will be given time to consider their participation and discuss the project and told that participation is voluntary. When a patient has had sufficient time to consider participation and they have agreed to take part they will be asked to sign a consent form. All screening procedures are described below in Section 7.

4.3 Inclusion criteria
Patients meeting all of the following criteria will be considered for admission to the study:
- Adults, either sex, ages 18-55 years or up to age 65 with < 20 pack year history
- Patients with a documented history of asthma for >6 consecutive months, and
- Patients presenting within 24 hours (of initial presentation to medical care) with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough and/or reduced PEF) and requiring a course of oral steroids
- Patients with a PEF less than 80% of predicted normal or patient’s best
- Patients must be able to complete diaries and quality of life questionnaires.
- Patients must sign and date an informed consent prior to any study procedures.

4.3 Exclusion criteria
Patients presenting with any of the following will not be included in the study:
- Smokers aged 56-65 with a >20 pack year history
- Patients requiring immediate placement in ICU
- Patients who used antibiotics within 28 days prior to enrolment
- Patients with known impaired hepatic function (ALT/AST > 2 ULN)
- Patients with significant lung disease (including COPD) other than asthma
- Patients with ≥ 10mg oral corticosteroid maintenance therapy
- Patients requiring other antibiotic therapy
- Patients who are receiving other medications or who have other disease conditions or infections that could interfere with the evaluation of drug efficacy or safety
- Women who are breast-feeding or are pregnant, as demonstrated by a urine pregnancy test carried out before exposure to study medication or the start of any study procedure that could pose a risk to the foetus
- Patients with suspected or known hypersensitivity to, or suspected serious adverse reaction to Azithromycin or any of the macrolide or ketolide class of antibiotics, erythromycin or to any excipients thereof
Patients who have received treatment with any other investigational drug within 1 month prior to study entry, or have such treatment planned for the study period during treatment and follow up phase

Patients with a concomitant condition (including clinically relevant cardiovascular, hepatic, neurologic, endocrine, or other major systemic disease) making implementation of the protocol or interpretation of the study results difficult

Patients with mental conditions rendering them unable to understand the nature, scope, and possible consequences of the study.

Patients unlikely to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits.

No subject will be allowed to enrol in this study more than once.

4.4 Withdrawal of patients

An early withdrawal visit will be performed on patients who are withdrawn from the study prematurely, at any time between receiving the first dose of study medication and visit 3, day 10.

Patients may be withdrawn from the study for the following reasons:

- At their own request or at the request of their legally authorised representative
- If, in the investigator’s opinion, continuation in the study would be detrimental to the subject’s well-being

Patients must be discontinued from study medication under the following circumstances:

- Pregnancy (every attempt must be made to follow-up patients who become pregnant to determine the outcome of the pregnancy)
- Deterioration of the clinical condition or delayed response—in the investigator's opinion—at least 48 hours after beginning study treatment; e.g., two doses of study medication
- Addition of any additional oral or parenteral antibiotic during study days 1-10
- The occurrence of severe unexpected reactions to the drug on more than one occasion

In all cases, the reason for withdrawal must be recorded on the case report form and in the subject's medical records. The subject must be followed up to establish whether the reason was an adverse event and, if so, this must be reported in accordance with the procedures in Section 8.

The investigator must make every effort to contact patients lost to follow-up.

4.5 Replacement of patients

Patients will not be replaced.
5. STUDY TREATMENTS

5.1 Details of study treatments

The following study treatments will be used in this study:

<table>
<thead>
<tr>
<th>Drug code:</th>
<th>Zithromax</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formulation:</td>
<td>Containing 500 mg of azithromycin (2×250mg capsules), over-encapsulated with lactose/magnesium stearate</td>
<td>Size 00 capsules containing lactose/magnesium stearate</td>
</tr>
<tr>
<td>Manufacturer:</td>
<td>Pfizer capsules, over-encapsulated by Bilcare</td>
<td>Bilcare</td>
</tr>
</tbody>
</table>

The identity of the treatment regimen will be blinded by encapsulating active medication in opaque (00) capsules to match the placebo.

5.2 Dosage schedule

All patients in the study will receive, per randomised allocation, treatment with either azithromycin or the placebo. Those randomised to azithromycin will receive 500 mg azithromycin (two 250 mg capsules) once a day for 3 days (this is the routine dose given in clinical care). Those patients randomised to the placebo will receive two placebo capsules once a day for 3 days. The duration of treatment with study medications will be 3 days. Patients will be instructed to take study medication at least 1 hour before or 2 hours after food and if they are taking antacids to take the study drug at least 1 hour before or 2 hours after the antacids.

The time of administration of the first and last doses of study medication, and the labeling on the study medication containers should be documented on the case report form. The first dose will be given in the presence of a member of the research team.

5.3 Treatment assignment

The study medication will be administered only to patients included in this study following the procedures set out in the study protocol.

All sites taking place in the study will be allocated a unique three digit site number. All patients who have signed an informed consent document will receive a three-digit subject number that will be used to identify the subject throughout the study. Subjects will be enrolled into the project using the online project InForm database and will be allocated their three-digit subject number sequentially as they are added to InForm so that no two subjects at the same site are allocated the same number. A patient can then be identified by their site number and subject number.
Randomisation will be web-based via access to a secure Imperial College server. Patient allocation will be stratified by centre performed in random length blocks.

Patients withdrawn from the study retain their subject number and their randomisation number, if already given. New patients must always be allotted a new subject number and, if applicable, a new randomisation number.

5.4 Blinding, packaging, and labeling

The identity of the study medications will be blinded and packaged according to the randomisation schedule and supplied to the investigator by Bilcare with code break envelopes. Over-encapsulated Zithromax capsules and placebo capsules will be placed into child resistant tamper evident containers and a randomised label applied to each container. When a patient is randomised into the study the details of the randomised label will be replicated in the patient’s study notes.

Emergency identification of study medication/unblinding

If it is medically imperative to know what study medication the subject is receiving, the investigator or authorised person should open the relevant code break envelope that corresponds to the randomisation label on the patients study drug container, exposing the blinded information. The investigator or the person who breaks the blind must record the date and the reasons for doing so in the case report form and in the subject’s medical records. The principal investigator must be contacted immediately to determine whether the subject should be withdrawn from the study.

The reason for unblinding should be assessed to determine if it is an adverse event and if so the reporting requirements in Section 8 should be followed.

5.5 Supplies and accountability

The pharmacist will inventory and acknowledge receipt of all shipments of study medication. All study medication must be kept in a locked area with access restricted to designated study personnel. The study medication must be stored in accordance with the manufacturer's instructions. The investigator or pharmacist will also keep accurate records of the quantities of study medication dispensed, used, and returned by each subject. The site monitor will periodically check the supplies of study medication held by the investigator or pharmacist to ensure accountability of all study medication used. At the conclusion of the study, all unused study medication and all medication containers will be returned to the hospital pharmacy for disposal unless other arrangements have been approved by the sponsor.

5.6 Compliance

The patients first return visit after being given the drug/placebo will be on day 5 at which point they should have completed the full dose of drug. Compliance will be assessed by capsule counts at this visit and unused study medication and all medication containers will be collected. Details will be recorded on the case report form.

5.7 Undesirable drug effects

Azithromycin is well tolerated with a low incidence of side effects. The following undesirable effects are noted in the summary of product characteristics:
Common effects (> 1/100, < 1/10)
• Gastrointestinal disorders – nausea, vomiting, diarrhoea, abdominal discomfort (pain/cramps)

Uncommon effects (> 1/1000, < 1/100)
• Nervous system disorders – dizziness/vertigo, somnolence, headache, convulsions, taste perversion, syncope
• Gastrointestinal disorders – Loose stools, flatulence, digestive disorders, anorexia, dyspepsia
• Skin and subcutaneous tissue disorders – allergic reactions including pruritus and rash
• Musculoskeletal, connective tissue and bone disorders – arthralgia
• Reproductive system and breast disorders – vaginitis

Rare effects (>1/10000, <1/1000)
• Blood and lymphatic system disorders – thrombocytopenia, occasional transient mild neutropenia
• Psychiatric disorders – aggressiveness, agitation, anxiety and nervousness
• Nervous system disorders – paraesthesia and asthenia, insomnia and hyperactivity
• Ear and labyrinth disorders – impaired hearing, deafness, ringing in the ears
• Cardiac disorders – palpitations, arrhythmias including ventricular tachycardia and rare reports of QT prolongation and torsades de pointes
• Vascular disorders – hypotension
• Gastrointestinal disorders – constipation, discolouration of the tongue, pancreatitis, pseudomembranous colitis
• Hepato-biliary disorders – hepatitis and cholestatic jaundice have been reported, including abnormal liver function test values, as well as rare cases of hepatic necrosis and hepatic dysfunction, which in rare instances have resulted in death
• Skin and subcutaneous tissue disorders – allergic reactions including angioneurotic oedema, urticaria and photosensitivity; serious skin reactions such as erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis
• Renal and urinary disorders – interstitial nephritis and acute renal failure
• General disorders – anaphylaxis including oedema (leads in rare cases to death), candidiasis, fatigue, malaise
6. PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 Prior and concomitant illnesses

Illnesses present at the time informed consent is given are regarded as concomitant illnesses and must be documented on the appropriate pages of the case report form.

Illnesses first occurring or detected during the study, and worsening of concomitant illness during the study, are to be regarded as adverse events and must be documented as such on the case report form (see Section 8).

6.2 Prior and concomitant treatments

All additional treatments being taken by the patients on entry to the study or at any time during the acute phase of the study are regarded as concomitant treatments and must be documented on the appropriate pages of the case report form.

Standard care treatment for asthma including bronchodilators, inhaled steroids, theophyllines, magnesium will be allowed.

6.2.1 Antibiotic treatments

No oral or parenteral concomitant antibiotic treatments are permitted for 28 days prior to randomisation, nor for the duration of study up to day 10. Patients receiving oral or parenteral antibiotic treatments that cannot be discontinued are not eligible for inclusion in the study, and patients requiring such antibiotic treatments other than the study medication during the treatment period and up to study day 10 must be withdrawn and the reasons noted on the case report form.

6.2.2 Nonantibiotic treatments

If concomitant nonantibiotic treatments are considered to be necessary for the subject's welfare and are unlikely to interfere with the study medication, they may be given at the discretion of the investigator.

6.2.3 Precautions

In patients taking concomitant antacids, azithromycin should be taken at least 1 hour before or 2 hours after the antacid. Patients will also be instructed to take study medication at least 1 hour before or 2 hours after food.

Interactions between macrolide antibiotics and the following drugs have been reported. If patients are receiving these medications, please monitor as per your standard clinical practice.

- theophylline
• cyclosporine
• carbamazepine
• cimetidine
• digoxin
• ergot derivatives
• methylprednisolone
• nelfinavir
• terfenadine
• zidovudine
• didanosine
• rifabutin
• coumarin-type oral anticoagulants including warfarin
7. STUDY PROCEDURES AND SCHEDULE

7.1 Overview of data collection

The primary efficacy analysis will be to demonstrate clinical efficacy of oral azithromycin (500 mg OD) treatment for 3 days in patients with acute exacerbations of asthma.

The primary assessment of clinical efficacy will be evaluated using the following criteria assessed 10 days after randomisation:

- Diary card summary symptom score

Additional efficacy variables will include:

- Health Status assessed by acute asthma QoLQ (Juniper)
- Health Status assessed by mini asthma QoLQ (Juniper)
- Pulmonary Function tests (FEV1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Time to 50% reduction in symptom score
- Primary and secondary outcomes to be assessed at days 5 and 10 post randomisation
- Trends in primary and secondary outcomes over the time course of the exacerbation up to 14 days
  - Assessment of the patient’s clinical improvement relative to initial
    - C. pneumoniae and/or M. pneumoniae status
    - standard bacteriologic status
    - virologic status
    - sputum inflammatory cell status

The assessment of safety will be performed using the following criteria:

- Adverse events

Additional assessment will include:

- Compliance with study medication, assessed by counting unused capsules at the end-of-therapy visit (visit 2, day 5)

7.2 Description of study days

Study visits can be +/- one day to avoid weekends (except for visit 1).

A summary of the Study Schedule is shown on page 8 above. The following observations will be made during the study according to the schedules below:

Visit 1 Day 1 (Pre-therapy/Entry visit – within 24 hours of initial presentation)
Inclusion/exclusion criteria
Conduct of study explained to patient and a signed informed consent obtained prior to the performance of any study procedures

- Demographics
- Medical/surgical history (per signed patient history or previous medical records) including documentation of:
  - asthma diagnosis
  - tobacco consumption
- Recording of time since presentation to medical care
- Assessment of severity of asthma at presentation using BTS guidelines on asthma severity
- Previous and concomitant treatment including previous antibiotic treatment
- Pulmonary function tests including FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅₋₇₅%, FEF₅₀%, and peak flow (See Section 7.3.1 Pulmonary Function Tests)
- Urine pregnancy tests for women of childbearing potential
- Serum for biomarkers and serology for atypical pathogens. (See Appendix C Instructions for collecting samples).
- Nasal swab/mucus for PCR for all common respiratory viruses
- Spontaneous or induced sputum for PCR for all common respiratory viruses and atypical bacterial pathogens. (See Appendix D).
- Spontaneous or induced sputum for cytoptins for cell differential and processing for sputum supernatant for mediators and biomarkers. (See Appendix D).
- Quantitative culture of sputum for standard bacteria
- Blood sample for full blood count
- Dispense patient diary and provide instructions for completing the diary (See 7.3.1 Patient’s Daily Recordings)
- Health outcomes assessment – Acute Asthma QoL (Juniper)
- Health outcomes assessment – MiniAQLQ (Juniper)
- Randomisation and study medication dispensed
- Instructions to bring all unused study medication to the next visit for verification
- Adverse event reporting requirements
- Appointment for next visit

Visit 2 Day 5 (End-of-therapy visit)

- Previous and concomitant treatment
- Pulmonary function tests FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅₋₇₅%, FEF₅₀%, peak flow (See Section 7.3.1 Pulmonary Function Tests)
- Diary review
- Health outcomes assessment – Acute Asthma QoL (Juniper)
- Health outcomes assessment – MiniAQLQ (Juniper)
• Collect and count unused drug
• Adverse event review
• Appointment for next visit

Visit 3 Day 10
• Previous and concomitant treatment
• Pulmonary function tests, FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅-₇₅%, FEF₅₀%, peak flow (See Section 7.3.1 Pulmonary Function Tests)
• Diary review
• Health outcomes assessment – Acute Asthma QoLQ (Juniper)
• Health outcomes assessment – MiniAQLQ (Juniper)
• Adverse event review
• Appointment for next visit

Visit 4 Day 42 (Follow-Up Visit)
• Serology for atypical pathogens. (See Appendix C Instructions for collecting samples).
• Adverse event review
7.3 Methods of data collection

7.3.1 Efficacy data

Pulmonary Function Tests (PFTs)

A spirometer that meets all ATS recommendations should be used. PFTs will be performed at Visits 1 to 3. PFTs will be measured three times in a consistent position (standing or sitting) throughout the study. The best FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅-₇₅%, FEF₅₀% and peak flow are to be recorded in the CRF as stated below:

1. Forced expiratory volume in one second (FEV₁) in litres;
2. Forced vital capacity (FVC) in litres;
3. Forced expiratory volume in one second/forced vital capacity (FEV₁/FVC) ratio;
4. Forced Mid-Expiratory Flow Rate (FEF₂₅-₇₅%) in litres/sec;
5. Forced Expiratory Flow Rate at 50% (FEF₅₀%) in litres/sec;
6. Peak expiratory flow (PEF) in litres/min

Patient's Daily Recordings

All patients will be supplied with a diary in which to record Salbutemol use, asthma symptom ratings and number of nighttime awakenings due to asthma symptoms. At Visit 1 patients will be instructed regarding recording of information in the diary (see below) and asked to complete the diary each day for 10 days. They will be reminded of the recording instructions at Visits 2 and 3.

I. Daytime symptom diary scale questions

1. How often did you experience asthma symptoms today?
   
   0  1  2  3  4  5  6
   None of the time      All of the time

2. How much did your asthma symptoms bother you today?
   
   0  1  2  3  4  5  6
   Not at all bothered      Severely bothered

3. How much activity could you do today?
   
   0  1  2  3  4  5  6
   More than usual activity      Less than usual activity

4. How often did your asthma affect your activities today?
   
   0  1  2  3  4  5  6
   None of the time      All of the time

II. Nocturnal diary scale question
1. Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning?)
   - No
   - Once
   - More than once
   - Awake “all night”

III. Number of inhalations of Salbutamol will be recorded in the diary. Each patient should be reminded that Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically.

IV. Study medication will be recorded in the diary. Any concomitant medication use will be recorded in the diary.

V. Adverse Events - patients will record all unusual health related events in the diary regardless of relationship to medication.

Clinical sample collection

Respiratory samples

- A nasal mucus sample or nasal swab will be taken at Visit 1 for PCR for viruses and atypical bacteria
- At Visit 1 in patients with a productive cough, deep expectorated sputum will be collected after rinsing the mouth with sterile water.
- In patients unable to produce an adequate sample of spontaneous sputum, sputum will be induced according to published protocols using isotonic saline.

The sputum will be processed for PCR, standard bacteriology and cytospin and supernatant production. If sputum cannot be obtained at visit 1 because of nonproductive cough or for any other reason, this must be documented on the case report form.

Serology

Acute (Visit 1) and convalescent (Follow up visit day 42) serum samples will be obtained, and forwarded to the Chief Investigator’s laboratory.

(see Appendix C Instructions for Collecting Samples, for details).

7.3.2 Safety data

Adverse events

Adverse events observed by the investigator or reported by the subject will be documented as described in Section 8.

7.3.3 Health outcomes data

Health outcomes will be measured to determine:

Overall assessment of symptom resolution during the first ten days based on global subject diary assessment

- Health Status at visits 1 to 3
  - Acute AsthmaQoLQ (Juniper)
  - Mini Asthma QOLQ (Juniper)
8. PHARMACOVIGILANCE

8.1 Definitions

8.1.1 Adverse Event (AE):

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.

The adverse event may be:

- A new illness
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness
- An effect of the study medication, including comparator
- A combination of two or more of these factors.

Variations in clinical observations are both common and expected consequences of the diseases of the upper respiratory tract and their resolution processes. An increase of these infection-related events may be seen in the subject population under study. They should be reported as adverse events when, in the opinion of the investigator, they deviate from normal in terms of frequency, intensity or duration. If the event meets the criteria of serious, then the event must be reported as a serious adverse event (see below).

8.1.2 Adverse Reaction (AR)

All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions.

8.1.3 Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable product information (ie the summary of product characteristics (SmPC) for azithromycin). Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.

8.1.4 Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Any untoward medical occurrence or effect that at any dose:

- Results in death
- Is life-threatening – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation
- Results in persistent or significant disability or incapacity – there is a substantial disruption of a person’s ability to carry out normal life functions
- Is a congenital abnormality or birth defect
Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

### 8.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any suspected adverse reaction related to an IMP that is both unexpected and serious.

### 8.2 Causality

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the Chief Investigator. Other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant's clinical condition, other concomitant treatment).</td>
</tr>
<tr>
<td>Possible</td>
<td>There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant's clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Probable</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>Definitely</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>Not assessable</td>
<td>There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.</td>
</tr>
</tbody>
</table>

### 8.3 Period of observation

For the purposes of this study, the period of observation extends from the time the subject gives informed consent until 7 days after the last dose of study medication.
8.4 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the sponsor in the first instance. A flowchart is given below to aid in the reporting procedures.

All adverse events that occur after the subject has signed the informed consent must be documented on the pages provided in the case report form. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the study medication.

All patients who have adverse events, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up.

8.4.1 Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form. These should be reported to the MHRA and REC on the annual safety report form to be completed on the anniversary of the date a favourable opinion for the study was given.

8.4.2 Serious AR/AEs/SUSARs

Fatal or life threatening SAEs and SUSARs should be reported to the Chief Investigator (who will report to the sponsor) on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). Additional information should be sent to the CI and sponsor within 5 days if the reaction has not resolved at the time of reporting.

SAEs
An SAE form should be completed and faxed to the Chief Investigator for all SAEs within 24 hours. The Chief Investigator should inform the sponsor of all SAEs within 24 hours of receiving notice of them. All SAEs should be recorded on the annual safety reports that are sent to the MHRA and REC on the anniversary of the date a favourable opinion for the study was given.

SUSARs
In the case of serious, unexpected and related adverse events, the staff at the site should:

Contact the Chief Investigator by phone and then complete the SAE case report form & send it immediately (within 24 hours, preferably by fax), signed and dated to the Chief Investigator together with relevant treatment forms and anonymised copies of all relevant investigations.

The Chief Investigator will notify the MHRA, the main REC and the sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and serious but non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.

Local Principal Investigators should report any SUSARs and /or SAEs as required by their Local Research Ethics Committee and/or Research & Development Office in addition to reporting them to the Chief Investigator.
Contact details for reporting SAEs and SUSARs
Fax: 0207 262 8913, attention Professor Sebastian Johnston
Please send SAE forms to: Professor Sebastian Johnston
Tel: 0207 594 3764 (Mon to Fri 09.00 – 17.00)
9. STATISTICAL PROCEDURES

9.1 Study populations

The intent-to-treat (ITT) population is to be used for statistical analyses:

9.1.1 Eligibility for clinical efficacy analysis

The following criteria will be applied:

Population of patients eligible for the ITT analysis:

- All randomised patients, as treated, who received at least one dose of study medication and with signs and symptoms of exacerbations of asthma.

9.1.2 Eligibility for bacteriological efficacy analysis

All ITT patients who are tested for *C. pneumoniae* and/or *M. pneumoniae* status, standard bacteriologic status, virologic status or sputum inflammatory cell/mediator status at baseline will be included for the bacteriological analyses.

9.1.3 Eligibility for safety analysis

All patients, as treated, who received at least one dose of the study treatment with post baseline safety assessment will be eligible for the safety analysis.

9.1.4 Major protocol violations

Patients who fall into any of the following categories will be classed as patients with major protocol violations:

- Wrong entry diagnosis
- Previously enrolled in the study
- Missing appropriate post-treatment information
- Received more than 24 hours of treatment with other antibiotics within 28 days prior to enrollment into the study (see Section 4.3 Exclusion criteria, page 16)
- Use of non study systemic antimicrobials between entry and day 10
- Insufficient treatment duration (to be defined in the analysis plan prior to unblinding). Any subject treated for at least 48 hours and judged by the investigator to be a clinical failure will be considered clinically evaluable.

Major protocol violations not limited to the above categories will be detailed in the final analysis plan prior to the unblinding of the database.

Deviations from clinical trial protocols and GCP occur commonly in clinical trials. The majority of these instances are technical deviations that do not result in harm to the trial subjects or significantly affect the scientific value of the reported results of the trial. These cases should be documented e.g. in the case report form for the trial or trial master file, in order for appropriate corrective and
preventative actions to be taken. In addition, these deviations should be included and considered when the clinical study report is produced.

Any serious breach of GCP or of the protocol is defined as one:

“which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial (this should be relevant to trial subjects in the UK); or

(b) the scientific value of the trial.”

It is the responsibility of the Sponsor to assess the impact of the breach on the scientific value of the trial. If it is decided that the breach is serious then it is the responsibility of the Principal Investigators to report it within 24 hours to the Chief Investigator and for the Chief Investigator to report within 24 hours to the sponsor. The Chief Investigator, in conjunction with the sponsor, should then submit a report of the breach to the MHRA within 7 days of becoming aware of the breach. This should be followed up by a full report when all details of the breach are known.

9.2 Statistical methods

A full Statistical Analysis Plan (SAP) will be developed and agreed by the Trial Steering Committee.

9.2.1 Analysis of efficacy data

The primary efficacy endpoint will be analysed using a 2-level hierarchical model (centre and patient as the two levels). The model will include treatment, study centre, the baseline value of the primary endpoint, and any predefined covariates. The treatment group means and the between group differences adjusted for any pre-specified covariates will be estimated using this model and the model will be used to test whether Azithromycin differs from placebo. The same hierarchical modelling will be used for all secondary endpoints.

Additionally, a hierarchical model at 3 levels (including day at the lowest level) will be fitted to allow an assessment of trends over time in primary and secondary endpoints.

9.2.2 Handling of missing data

A missing data strategy will outlined in the SAP using multiple imputation to acknowledge the associated uncertainty.

The covariates include severity of asthma; severity of exacerbation as measured by % predicted FEV and % predicted PEF at recruitment; age; gender; acute AQLQ and mini AQLQ (Juniper) at recruitment; smoking status; atypical bacterial positive status (serology or culture or PCR positives); standard bacterial status (sputum positive or negative); virus PCR positive status; seasonality; exploratory and sensitivity analyses will examine the effects of these predictors and their possible interactions with treatment effect.

The predefined limited number of subset analyses will be specified in the detailed Statistical Analysis Plan.

9.2.3 Analysis of safety data

Adverse events
Frequencies of patients with treatment-emergent adverse events, regardless of relationship to study treatment and sorted by body system, will be summarised by treatment group. Frequencies of patients with possible treatment-related adverse events will also be displayed. Frequencies of patients with adverse events including those leading to death or permanent discontinuation of study medication will be likewise summarised. Frequency tables of adverse events, displayed by intensity, will also be provided.

**Exploratory analyses**
Regression models will be used to analyse each of the exploratory questions. These will be analogous to those described above for the primary and secondary outcomes.

### 9.3 Sample size justification

The sample size calculations are based on the primary outcome: Change from baseline in diary card summary asthma symptom scores at 10 days after randomisation. Our previous study\(^1\) found the mean decrease in symptom score of 1.3 in treatment group, and 1 in the control group, resulting in the difference of -0.3 (SD 0.783) between the groups at 10 days.

Using a two-sided t-test at 1% significance level with 80% power we would need 161 patients in each group to be able to detect the same difference in asthma scores between the groups. The significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable, as well as to provide greater power for the subgroup exploratory analyses, as those subgroup analyses that were performed were uninformative in the 280 patient Telicast study\(^1\).

Taking into account the drop-out rate of 15% in the study\(^1\), we propose to recruit 190 patients in each arm of the study. To be able to run the trial within the project timelines, we intend to involve 10 centres.
10. ETHICAL AND LEGAL ASPECTS

10.1 Good Clinical Practice (GCP)

“Good clinical practice is a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects.” - EU Directive 2001/20/EC, article 1, clause 2. Compliance with this good practice provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible and accurate.

The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abides by the principles of good clinical practice (GCP) as described in articles 2 to 5 in the EU Directive 2005/28/EC as well as in the ICH Harmonised Tripartite Guidelines Topic E 6: “Guideline for Good Clinical Practice.” Compliance with these regulations also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki (October 2008). The study will also be carried out in keeping with local legal and regulatory requirements.

10.2 Delegation of investigator responsibilities

The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The investigator should maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

10.3 Subject information and informed consent

Before being admitted to the clinical study, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her.

An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. The document must be in a language understandable to the subject and must specify who informed the subject. Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Consent can be taken by any Clinician or Clinical Research Nurse working on the project.

After reading the informed consent document, the subject must give consent in writing if they agree to participate in the study. The subject's consent must be confirmed at the time of consent by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussions. If the subject is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to patients must take place in the presence of an impartial witness. Consent must be confirmed at the time of consent orally and by the personally dated signature of the subject or by a local legally recognised alternative
(e.g., the subject's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document.

A copy of the signed consent document must be given to the subject. The original signed consent document will be retained by the investigator and a copy placed in the patients hospital medical record.

The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

The investigator will inform the subject’s General Practitioner (GP) about the subject’s participation in the trial if the subject has a GP and if the subject agrees to their GP being informed.

Subjects are free to withdraw from the study at any time, without giving a reason. This would not affect the standard care they receive.

10.4 Trial Governance

This trial will be sponsored by Imperial College London and the Imperial Clinical Trials Unit (ICTU) will be responsible for the coordination. ICTU provides experienced staff within an infrastructure supporting the management, monitoring and reporting of clinical trials involving investigational medicinal products (IMP’s). ICTU has systems in place to ensure compliance with the Medicines for Human Use (Clinical Trials) Regulations 2004.

Adverse Events will all be notified to the Data Monitoring and Ethical Committee as well as being reviewed by the Trial Management Committee who will respond accordingly.

This study will not open to recruitment until appropriate approvals and authorisations have been obtained from an independent ethical committee and the Medicines & Healthcare Products Regulatory Agency. Recruitment will not commence at an individual participating site until local NHS Management approval has been obtained and all local documentation is in place and all requirements have been fulfilled according to ICTU Standard Operating Procedures (SOPs).

The plans for the Governance of this trial include the involvement of the ICTU Quality Assurance (QA) Manager. The QA Manager will oversee the creation of all essential documentation to be used in this trial, which will be written with reference to the ICTU SOPs to ensure they comply with all ethical and regulatory requirements and are fit for purpose. The QA manager will also conduct a Risk Assessment on the study to ensure the development of an appropriate monitoring plan, which takes into account the particular requirements of this study. Monitoring will be implemented accordingly under the supervision of the Trial Manager. Oversight of the trial will be conducted on a day-to-day basis by the QA Manager and the Director of Operations.

Trial Documentation

ICTU carries out all its trials in compliance with its SOPs. These cover all aspects of setting up and managing multi-centre clinical trials. For example, they include templates for writing protocols, consent documents, DMEC charters and reports. They also include checklists to ensure activities eg the release of IMPs to the investigator sites, are conducted appropriately. Essential documents will also be created, collected and housed in accordance with ICH (E6) GCP Guidelines section 8.2 and regulatory requirements. A trial master file will be established at ICTU as directed by the sponsor and corresponding site files will be established at each investigator site. The trial shall not begin before all relevant documentation has been collected, distributed and verified by the monitor(s)/ trial
management at or before site initiation as necessary. Similarly, trial close-out shall not be performed before the monitors(s)/ trial management has reviewed investigator and ICTU files and verified that all necessary documents are in the appropriate files. Essential documents will be available for inspection by auditors, regulatory inspectors and study monitors.

Retention of Documents

ICTU has in place well established protocols for the protection of data and for retention of documents. Data will be stored for a minimum of 15 years (or according to changes in regulatory requirements) following completion of this trial. Data generated by this work will be processed in accordance with the Data Protection Act 1998. ICTU will adhere to the Imperial College Code of Practice, drawn up in association with the Imperial’s Data Protection Policy, relating to the collection, holding and disclosure of data relating to individuals. The Principal and Co-applicants will act as custodians of the data, and be responsible for its security.

The PI at each investigational site is responsible for the archiving of all the essential trial documents, including the Investigator Site File, in accordance with regulatory requirements. The PI will ensure the continued storage of the documents, even if s/he were to leave the clinic/practice or retire before the end of the required storage period. Delegation of these responsibilities will be documented in writing.

Trial Management

The Trial Steering Committee will have an independent chair and will also include a patient representative and the lead investigator as well as additional committee members from the project team. A Data Monitoring & Ethical Committee will also be established, and options for suitable independent candidates to chair this committee are also being explored. The Trial Management Committee will be chaired by Professor Sebastian Johnston and will be made up of the Principal Investigators (PI’s), PI’s, the Study Manager, a study Statistician and representative from ICTU.

10.5 Confidentiality

Only the subject number and subject initials will be recorded in the case report form, and if the subject name appears on any other document (e.g., pathologist report) it will be completely anonymised.

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

10.6 Approval of study protocol amendments

The REC, MHRA, R&D office and sponsor must be informed of all subsequent protocol amendments. Amendments must be evaluated to determine whether or not they are substantial and therefore if formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communication with the REC and, if applicable, between study investigators and the REC. This also applies to any communication between the investigator (or coordinating investigator, if applicable) and the authorities.
10.7 Monitoring and audit

The Principal Investigators will permit trial related monitoring, audits, ethics review and regulatory inspections, providing direct access to source data/documents as required. The project will be monitored by the project monitor and subject to the audit and monitoring requirements of Imperial College, London and all NHS sites at which the project is taking place.

10.8 Publication Policy

All publications and presentations relating to the study must be authorised by the Chief Investigator.
11 DOCUMENTATION AND USE OF STUDY FINDINGS

11.1 Documentation of study findings

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the electronic case report form using the InForm database. Training will be provided to all project staff on the use and completion of the InForm database and a database user guide will be available. The investigator, or designated representative, should complete the case report form pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

A source data location list will be prepared and updated during the study. This list will be filed in both the trial master file and the investigator study file.
12. STUDY DURATION AND DATES

The study will start June 1st 2011 for development of CRFs and training of personnel. Subject recruitment is proposed to last 1 year to start 1st September 2011 and end in August 2012. Laboratory analyses, data cleaning, analysis and reporting will take a further 9 months.

12.1 Definition of the end of the trial

The date of the last visit of the last patient undergoing the trial.
13. REFERENCES


Appendices

APPENDIX A...INSTRUCTIONS FOR ADMINISTRATION OF THE AQLQ.................................................................44
APPENDIX B  PATIENT DIARY ..................................................................................................................................51
APPENDIX C  INSTRUCTIONS FOR COLLECTING AND ANALYSIS OF SAMPLES .............................................................................................................52
APPENDIX D  INDUCED SPUTUM PROCESSING PROTOCOL.................................................................57
APPENDIX A INSTRUCTIONS FOR ADMINISTRATION OF THE AQLQ

If possible, the AQLQ should be the first questionnaire completed during a clinic visit and should precede any discussion with a health professional. If patients discuss their health state before completing the questionnaire, the response of the health professional to the patient’s experiences may influence how the patient answers the questionnaire.

General Instructions for Questionnaire Administration

It is important to ensure that patients understand the questionnaires, so they can provide quality information throughout the study. The Acute Asthma Quality of Life Questionnaire (Acute AQLQ) is designed to find out how the patient has been feeling during the last half hour and the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) has been designed to find out how the patient has been feeling during the last 3 days.

Both the Acute AQLQ and the MiniAQLQ should be interviewer administered. Study coordinators should avoid interpreting questions for patients, and encourage them to interpret questions as best they can and then answer accordingly.

It is very important that none of the questionnaires are administered to patients prior to signing the consent form, just as with any other clinical data you are collecting. Most patients will have little or no experience of completing questionnaires of this sort, so the study coordinator’s explanations and enthusiasm are important.

Interviewer Administered Questionnaires

Staff who will be administering the questionnaires should read the ‘Background, Administration and Analysis’ for all AQLQs before administering any questionnaires. The key points to remember are:

• Ask the questions exactly as worded
• Never help a patient choose a response
• Emphasise that there are no right or wrong answers
• Be neutral to the patient’s responses
• Ask all questions in the order specified
• Avoid missing data – it is vital that all questions have been answered

Answers to Patient Questions

Occasionally, a patient will express concern about his or her ability to follow these directions. There are several standard concerns patients may express:

“Should I say how I feel now, or how I’ve felt since the last visit?”

Patients should be told to respond for the period as indicated for each questionnaire i.e for the last half hour for the Acute AQLQ and the last 3 days for the MiniAQLQ. If they report that they feel differently today compared to the last 3 days, ask them to consider how they have felt “on average” or “most of the time” over the previous 3 days.

“That question doesn’t apply to me.”

Occasionally, a patient will remark that a particular item on the questionnaire seems directed at someone else. For example, they may not engage in certain activities such as walking up
stairs. Ask them to put down their best guess based on what they think would happen if they did try to engage in that activity. A patient’s perception that an item “does not apply” is not a valid reason for missing data. It’s far better for them to estimate than leave it blank.

Confidentiality is a concern of some patients. Whenever a questionnaire is handed to the patient, the study coordinator should reassure the patient that “all information is strictly confidential and does not become a part of your general medical record. The physician will not review this information to make treatment decisions”. Tell concerned patients of specific steps taken to protect their privacy such as referring to their records by a study identification number, not a name, and the reporting of results for groups, not individual patients. If this is confusing, tell them this research is like reporting an average grade for the class, rather than posting each student’s grade.

Problems of understanding questions should be minimal but if a patient states they do not understand the administrator should never attempt to reword or paraphrase the question. The administrator should initially repeat the question then if this does not help the correct response is “Whatever it means to you” emphasising there are no right or wrong answers.

**Specific Instructions for AQOLQ Administration**

The Asthma Quality of Life instrument asks patients about symptoms, emotions, activities and environmental factors related to asthma. All of the response scales have seven options that range from no effect to a constant effect on their lives.
Sample Acute AQLQ

This questionnaire has been tested using the wording and format that follows. It is important that interviewers adhere to the exact wording when addressing patients (regular type) and follow the instructions (italics). Deviation from both wording and instructions may impair the validity of the questionnaire.

This questionnaire has been designed to find out how you have been feeling during the last half hour (30 minutes). You will be asked about your symptoms and how your asthma has made you feel.

Show the red and yellow cards to the patient and explain the scales.

Make sure that he/she has the correct card for each question and that he/she reads it before responding.

Remind the patient that you only want to know about how he/she has been during the last half hour.

Record the patient’s answers on the response sheet.

I want you to tell me how much you have been bothered by your asthma during the last half hour.

1. How bothered have you been by shortness of breath during the last half hour? (Red Card)
2. How bothered have you been by coughing during the last half hour? (Red Card)
3. How much of the time have you felt afraid of not having your asthma medications available during the last half hour? (Yellow Card)
4. How much of the time have you experienced a feeling of fighting for air during the last half hour? (Yellow Card)
5. How much of the time have you felt frustrated as a result of your asthma during the last half hour? (Yellow Card)
6. How much of the time have you felt **concerned about the need to use medications for your asthma** during the last half hour? *(Yellow Card)*

7. How bothered have you been by **chest tightness or chest heaviness** during the last half hour? *(Red Card)*

8. How bothered have you been by **wheezing** during the last half hour? *(Red Card)*

9. How bothered have you been by **difficulty breathing out** during the last half hour? *(Red Card)*

10. How much of the time have you felt **afraid of getting out of breath** during the last half hour? *(Yellow Card)*

11. How much of the time have you felt **concerned about having asthma** during the last half hour? *(Yellow Card)*

### RESPONSE SHEET

<table>
<thead>
<tr>
<th>RESPONSE</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. shortness of breath</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>2. coughing</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>3. afraid of not having your asthma medications available</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>4. fighting for air</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>5. frustrated</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>6. concerned about the need to use medications for your asthma</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>7. chest tightness or chest heaviness</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>8. wheezing</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>9. difficulty breathing out</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>10. afraid of getting out of breath</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
<tr>
<td>11. concerned about having asthma</td>
<td>.....</td>
<td>.....</td>
<td>.....</td>
</tr>
</tbody>
</table>
RESPONSE OPTION CARDS

RED CARD

1. Extremely Bothered
2. Very Bothered
3. Quite Bothered
4. Somewhat Bothered
5. Bothered a bit
6. Hardly Bothered at all
7. Not bothered

YELLOW CARD

1. All of the time
2. Most of the time
3. Quite often
4. Some of the time
5. Once in a while
6. Hardly any of the time
7. None of the time
# Sample MiniAQLQ

**MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE**

<table>
<thead>
<tr>
<th>PATIENT ID</th>
<th>DATE</th>
</tr>
</thead>
</table>

**SELF-ADMINISTERED**

**Please complete all questions by circling the number that best describes how you have been during the last 2 weeks as a result of your asthma.**

**IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:**

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>Hardly Any of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Feel SHORT OF BREATH as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>2. Feel bothered by or have to avoid DUST in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>3. Feel FRUSTRATED as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4. Feel bothered by COUGHING?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>5. Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>6. Experience a feeling of CHEST TIGHTNESS or CHEST HEAVINESS?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>7. Feel bothered by or have to avoid CIGARETTE SMOKE in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>8. Have DIFFICULTY GETTING A GOOD NIGHT’S SLEEP as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>9. Feel CONCERNED ABOUT HAVING ASTHMA?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>10. Experience a WHEEZE in your chest?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

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MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE

SELF-ADMINISTERED

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

<table>
<thead>
<tr>
<th>Activity</th>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>Hardly Any of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>11. Feel bothered by or have to avoid going outside because of WEATHER OR AIR POLLUTION?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS DOING THESE ACTIVITIES AS A RESULT OF YOUR ASTHMA?

<table>
<thead>
<tr>
<th>Activity</th>
<th>Totally Limited</th>
<th>Extremely Limited</th>
<th>Very Limited</th>
<th>Moderate Limitation</th>
<th>Some Limitation</th>
<th>A Little Limitation</th>
<th>Not at all Limited</th>
</tr>
</thead>
<tbody>
<tr>
<td>12. STRENUIOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>13. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>14. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>15. WORK-RELATED ACTIVITIES* (tasks you have to do at work)</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>

*If you are not employed or self-employed, these should be tasks you have to do most days.

DOMAIN CODE:

- Symptoms: 1, 4, 6, 8, 10
- Activity Limitation: 12, 13, 14, 15
- Emotional Function: 3, 5, 9
- Environmental Stimuli: 2, 7, 11
APPENDIX B PATIENT DIARY

All patients will be supplied with a diary in which to record Salbutamol use, asthma symptom ratings and number of nighttime awakenings due to asthma symptoms. At Visit 1 patients will be instructed regarding recording of information in the diary (see below), and reminded of the recording instructions at Visit 2.

I Daytime symptom diary scale questions

1. How often did you experience asthma symptoms today?
   0  1  2  3  4  5  6
   None of the time  All of the time

2. How much did your asthma symptoms bother you today?
   0  1  2  3  4  5  6
   Not at all bothered  Severely bothered

3. How much activity could you do today?
   0  1  2  3  4  5  6
   More than usual activity  Less than usual activity

4. How often did your asthma affect your activities today?
   0  1  2  3  4  5  6
   None of the time  All of the time

II Nocturnal diary scale question

1. Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning?)
   □ No     □ Once     □ More than once     □ Awake “all night”

III Number of inhalations of Salbutamol will be recorded in the diary. Each patient should be reminded that Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically.

IV Study medication will be recorded in the diary. Any concomitant medication use will be recorded in the diary.

V Adverse Events - patients will record all unusual health related events in the diary regardless of relationship to medication.
APPENDIX C. INSTRUCTIONS FOR COLLECTING AND ANALYSIS OF SAMPLES

Sample collection

Sputum samples for standard bacterial culture, PCR for common respiratory viruses and atypical bacterial pathogens and for cytospins and sputum supernatant preparation

Spontaneous sputum sample should consist of expectorated sputum produced from a deep cough and collected as follows:

- Patients should rinse the mouth and gargle with water prior to sputum collection.
- Collect deep cough specimen into a sterile Petri dish. Instruct patients not to expectorate saliva or postnasal discharge into the container.

Induced sputum will be induced according to the following protocol:

Sputum induction will only performed if subjects can perform an adequate forced expiratory volume in one second (FEV₁) or peak flow manoeuvre, and if this is >25% of the predicted value after pretreatment with salbutamol 200µg delivered via Volumatic spacer. All subjects will receive supplemental oxygen during sputum induction. Sputum will be induced using sterile normal saline (0.9%) delivered from an ultrasonic nebuliser such as ULTRA-NEB®; DeVilbiss Health Care Inc., Somerset, PA, USA with a Hans Rudolph 2700 two-way nonrebreathing valve box (Hans Rudolph, Inc., Kansas City, MO, USA), as described previously. Spirometry will be performed 1 min after each nebulisation period, and supplemental salbutamol administered if there is a fall of ≥20% in spirometric results from baseline. Sputum induction will be stopped once an adequate sample is obtained, if spirometric results fall below 20% of baseline and do not recover within 10min, at the subject's request or at the investigators' discretion as described previously.

For both spontaneous and induced sputum:

- Take 1 medium-large sized plug for standard microbiology and transport the specimen to the local microbiology laboratory for bacteriologic processing immediately.
- Take 1 small-medium sized plug for PCR and place into a sterile 5mL tube labeled with the study site number, subject number, subject initials, and the date the sample was collected. Store at ≤80°C.
- Process the remaining sputum for preparation of cytospins and sputum supernatant as per Appendix D below.

Nasal swab/mucus for PCR for common respiratory viruses and atypical bacterial pathogens

- If the patient has rhinorrhoea take a nasal mucus sample using a clean soft tissue as described below.
- If the patient has no rhinorrhoea take a nasal swab as described below.
Patient's instructions for nasal mucus collection in tissue paper for PCR

1. Blow your nose from both nostrils into the tissue paper (preferably using a gentle tissue and not kitchen roll or paper towel).

2. Put the tissue paper in the provided plastic bag and close with the zip lock

3. Write on the plastic bag: study site number, subject initials and date and time sample has been taken using the permanent marker pen

4. Place the plastic bags into a labeled box suitable for -80 storage

5. Member of research team to put the box in to the -80 freezer for storage

Nasal swab collection for PCR

Procedure:

1. Put on mask and gloves

2. Insert (or ask the patient to insert) the swab slowly and very gently into one nostril, going straight back (not upwards). Continue along the floor of the nasal passage for 2 to 3 centimetres. Leave the swab in place to absorb mucus for 2 to 3 minutes.

3. Rotate the swab gently for 5-10 seconds to loosen the epithelial cells

4. Remove swab slowly and gently and immediately insert into the bijoux with PBS or normal saline

5. Bend or clip the wire swab handles to fit in the bijoux and reattach the cap securely.

6. Label the sample with the study site number, subject number, subject initials, and the date the sample was collected

7. The specimen should be held and transported at refrigerator temperature (4°C) and delivered to the laboratory as soon as possible for storage at -80°C.

Serum samples for serology and biomarkers

Serum samples (10 mLs) for serologic testing including detection of antibodies to M. pneumoniae and C. pneumoniae will be collected at visit 1 and the Follow up visit at day 42

Processing Serum

1. 10ml blood will be collected into one 10ml red capped blood collection tube.

2. Leave to clot for 30-40 mins at room temperature. The blood collecting tube should be protected from light by covering with aluminium foil throughout the whole of this incubation.

3. Spin for 10 mins at 4C at 2500xg or 3500rpm

4. You can normally obtain 3-4ml of serum from 10ml blood, therefore label 16 tubes.

5. Transfer the serum (Pale yellow upper layer) into a 15ml centrifuge tube using a sterile pasteur pipette-take care not to contaminate with any blood cells.

6. Mix serum by inventing capped 15ml centrifuge tube 5 times.

7. Pipette 250mkl aliquots into 0.5ml amber tubes with O-ring seals using a 1ml pipette with a barrier tip. Cap with green lid
8. Store aliquots immediately at -70°C freezer

**Sample processing**

**Microbiology**

**Sputum:**

- 0.2ml of homogenised sputum will be delivered to Bacteriology in a sterile container. This is a 1:2 dilution of sputum.
- Inoculate 10µl of the homogenate onto Blood agar+optochin disc, chocolate agar, CLED agar and Sabouraud agar and spread with a loop for discrete colonies.
- Take 100µl of the homogenate and add to 5ml sterile saline and mix, this is now a 1:100 dilution.
- Take 50µl of the 1:100 dilution and add to 5ml sterile saline, the final dilution being 10^-4.
- Inoculate 20µl of the 10^-4 dilution onto a blood and chocolate agar and spread over the entire surface with a loop.
- Incubate the blood and chocolate agars at 37°C in CO2, the CLED and Sabouraud agars at 37°C.

**Interpretation of the 10^-4 dilution cultures.**

<table>
<thead>
<tr>
<th>Colonies</th>
<th>CFU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>10^6</td>
</tr>
<tr>
<td>2-20</td>
<td>10^7</td>
</tr>
<tr>
<td>20-200</td>
<td>10^8</td>
</tr>
<tr>
<td>&gt;200</td>
<td>10^9</td>
</tr>
</tbody>
</table>

Only significant pathogens need to be counted and followed up with the appropriate identification and sensitivity.

Normal respiratory flora should be recorded as normal

**Transport of Specimens**

Packaging details, and any additional information necessary for shipping samples will be provided to each site, and the samples should be shipped by express mail on Monday through Thursday to the address below:

Dr Tatiana Kebadze  
Department of Respiratory Medicine  
National Heart & Lung Institute  
Norfolk Place  
London, W2 1PG  
United Kingdom  
Phone: 020 7594 3853  
FAX: 020 72628913  
t.kebadze@imperial.ac.uk
Laboratory analysis of samples

Serology for Atypical Bacteria
Acute and convalescent serum samples will be obtained for determination of titers of antibodies to *M. pneumoniae* and *C. pneumoniae*. IgM, IgG, and IgA antibodies against *C. pneumoniae* will be detected by:

- *C. pneumoniae* microimmunofluorescence (MIF; Focus Technologies, Cypress, CA, USA) and
- Medac *C. pneumoniae* sandwich enzyme-linked immunosorbent assay (ELISA; Medac, Hamburg, Germany)

Serologic diagnosis of *M. pneumoniae* infection will be performed using:

- *M. pneumoniae* particle agglutination titers (Serodia-Myco II, Fujirebio Inc., Japan) and
- *M. pneumoniae* IgM ELISA (Serion, Germany)

In addition

Acute and convalescent sera will be tested for *C. pneumoniae* IgG and IgA using ELISA (Biocline Pty Ltd, Sydney, Australia). A test index calculated from optical densities relative to that of control material will be used, where an index of <0.9 is negative, 0.9–1.09 is equivocal, 1.10–2.99 is positive and >2.99 is strongly positive. A rise in antibody response will be regarded as a change in index of ≥1.35 for *C. pneumoniae* IgG and ≥1.0 for IgA, which corresponds to a four-fold rise in titre by microimmunofluorescent assay.

*C. pneumoniae* and *M. pneumoniae* infection will be diagnosed by the presence of IgM serum antibodies and/or a fourfold rise between baseline and convalescent samples in IgG (*C. pneumoniae*) or particle agglutination titer (*M. pneumoniae*) as previously described and/or a positive sputum or nasopharyngeal PCR.

PCR of nasal and sputum samples for viruses and Atypical Bacteria

Viruses and atypical bacteria will be detected by PCR on random hexamer primed cDNA for *M. pneumoniae* and *C. pneumoniae*, rhinoviruses and other picornaviruses, adenoviruses, bocavirus, respiratory syncytial virus, influenza AH1/AH3/B, parainfluenza viruses 1–3, human metapneumoviruses (HMPV), and coronaviruses 229E and OC43 using established in house methodology as described, except bocavirus, which will be detected on 2μL random primed cDNA by PCR primers HBOV 01.2 TAT-GGC-CAA-GGC-AAT-CGT-CCA-AG and HBOV 02.2 GCC-GGC-TGA-ACA-TGA-GAA-ACA-GA with the following cycling conditions 94°C, 56°C and 72°C each for 20 secs for 35 cycles. Positive control is complete coding genome of bocavirus plasmid DNA.

In addition we will perform the following commercial assays for atypical bacteria:
The MutaPLATE® real time (TaqMan) PCR Kit Qualitative assay for the specific detection of *C. pneumoniae* in clinical samples of the respiratory tract using real time PCR microplate systems (e.g. Applied Biosystems / Stratagene or Corbett Research) from Immunodiagnostik, Bensheim, Germany and:

The Venor® Mp-QP *M. pneumoniae* detection Kit for real-time PCR from Minerva Biolabs GmbH, Berlin, Germany.
APPENDIX D. INDUCED SPUTUM PROCESSING PROTOCOL

DOCUMENT OWNER: CHRIS BRIGHTLING…..VERSION 1.1…..DATE: 16TH MAY 2008

1. Personnel

All personnel involved in processing sputum samples must be appropriately qualified laboratory trained staff with experience conducting this procedure.

2. Experimental

2.1 Materials required for sputum processing

The following materials and equipment will be used. If alternative consumables are used, they must be of equivalent specification this should be previously agreed by the study team.

1) Sputolysin from Calbiochem (Code 560000)
2) Dulbecco’s Phosphate-buffered saline (PBS) from Sigma (Code D-8662)
3) 48µm nylon filter gauze from Precision textiles, Bury.
4) Sarstedt polypropylene 15mL centrifuge tubes with conical base (Cat 62.554.00)
5) Funnels, Kartell polyprolylene powder funnels from VWE International (Cat 237/0230/04)
6) Sterile Petri dishes 90mm diameter from Fisher (Cat FB51508)
7) Sarstedt polypropylene 2 mL tubes with skirted base (Cat 72.694)
8) Shandon III cytocentrifuge and Shandon disposable cytofunnels (Cat A78710003)
9) Neubauer haemocytometer, rhodium coated. Fisher (Cat MNK-420-010N) (One for each clinic).
10) Trypan Blue 0.4% from Sigma (Cat T8154)
11) 1.5mL Eppendorf tubes from Fisher (Cat FB56023)
12) Featherweight tweezers Fisher (Cat DKC-621-Y) and Curved fine point forceps. Fisher (Cat DKC-440-Q)
13) Polysine microscope slides. Fisher (Cat MNJ-800-010F) or also available from Thermoshandon
14) Spiromix (or similar). Fisher (Cat MPR-455-L)
15) -20°C (or lower) freezer.
16) Benchtop refrigerated centrifuge. Heraeus Megafuge. Fisher (Cat CFH-170-020H) or similar
17) Electronic balance with readability of 0.001g. Fisher (Cat FB51988) or similar
18) Supply of crushed ice
19) Sterile water
20) 3mL Pasteur pipettes. Fisher PMK-400-05 1L or similar.
21) Slide mailers. Fisher FB70908 or similar.

2.2 Sputum Processing Worksheet

All data generated during sputum processing must be recorded on the Sputum Processing Worksheet.

2.3 Sputum Processing Method
Sputum is collected on ice and processed at 4°C within 2 hours of expectoration (Figure 1). Procedures 1-5 below must be performed on ice.

1. Empty whole sample into a petri dish and place on a black background. Select sputum plugs from saliva, using fine forceps and transfer to petri dish lid (if necessary using inverted microscope). Using larger blunt ended forceps gather the sputum plugs into one mass then condense it by moving around the lid with small circular motions. The aim is to spread saliva across the lid but to keep sputum in one mass.

   Note: Where the mass is very large e.g. CF samples, it is recommended that no more than approximately 1.5g of plug weight is processed.

2. Transfer sputum free from salivary contamination using blunt forceps into an empty (pre-weighed) polypropylene centrifuge tube (opaque) with screw top. (Do not use polystyrene tubes as they cause cell adhesion).

3. Subtract the weight of the empty centrifuge tube from the weight of the centrifuge tube plus selected sputum to obtain the weight of sputum portion to be processed.

4. Add 8 volumes x sputum weight (in grammes) of Dulbecco's phosphate buffered saline (D-PBS).

5. Disperse sputum by repeated gentle aspiration into a plastic pipette, vortex for 15 seconds and 15 minutes rocking on a bench rocker on ice.

6. Centrifuge at 790 x g for 10 minutes at 4°C (brake off).

7. Remove 4 volumes of the supernatant into a fresh centrifuge tube.

8. Centrifuge supernatant at 1500 x g for 10 minutes at 4°C (brake off).

9. Sub-aliquot the supernatant as specified per study. Apply a label to each micro tube and fill in site code, subject code, patient initials and randomisation code. Complete the requisition form by recording the site code, subject doe, patient initials, visit number and randomisation code. Place all micro tubes for an individual patient into a freezer bag (together with the requisition form) and freeze at -70°C to –80°C.

10. Freshly prepare 0.2 % Sputolysin (DTT) by diluting 10 mL (1 ampoule) of sputolysin with 40 mL of sterile water.

11. Add four volumes (weight of selected sputum) of 0.2% sputolysin to selected sputum pellet (i.e. 4 mL 0.2% sputolysin per gram of selected sputum weight determined in stage 3).

12. Disperse sputum by repeated gentle aspiration into a plastic pipette, vortex for 15 seconds and 15 minutes rocking on a bench rocker on ice.
13. Vortex for a further 15 seconds, filter the sputum suspension through 48\(\mu\)m nylon gauze placed in a funnel, pre-wet filter with D-PBS, shake off excess. Filter into a clean pre-weighed 15mL centrifuge tube, weigh the tube plus filtrate and note the filtrate weight on the worksheet.

14. Assess total cell count and cell viability using a Neubauer haemocytometer and the trypan blue exclusion method:

- Flood haemocytometer with 10 \(\mu\)L of cell filtrate mixed thoroughly with 10 \(\mu\)L of 0.4% trypan blue [dilution=2]), after which, the cells should be counted within 5 minutes.

- Count all cells in the centre square and in the four 1mm corner squares of chamber 1 of the haemocytometer. Cells should be classified as viable, non-viable and squamous (whether viable or not). At the investigator training sessions, the centre staff will be taught how to determine this, using the Trypan Blue exclusion method. Cells touching top and left lines are counted, cells touching lower and right lines are not. Calculate the mean number of cells per square and the percentage of viable leukocytes and squamous cells.

- Calculate the total number of cells and the total cell count (cells/g selected sputum).

\[
\text{Total number of cells (x 10^6)} = \frac{\text{mean number of cells/square x 2 x volume of cell filtrate in (mL)}}{100}
\]

\[
\text{Total cell count (cells x 10^6/g sputum)} = \frac{\text{mean number of cells/square x 2 x volume of cell filtrate in (mL)}}{100 \times \text{weight of selected sputum (g)}}
\]

15. Centrifuge filtered sample at 2000rpm (790 x \(g\)) for 10 minutes (brake off).

16. Aliquot supernatant into 2 mL micro tubes, leaving behind a covering of fluid and the undisturbed pellet. Apply a label to each micro tube and fill in site code, subject code, patient initials and randomisation code. Complete the requisition form by recording the site code, subject code, patient initials, visit number and randomisation code. Place all micro tubes for an individual patient into a freezer bag (together with the requisition form) and freeze at -70\(^\circ\)C to –80\(^\circ\)C.

17. Resuspend the cell pellet in a small volume of D-PBS and then adjust the cell suspension to 0.5 x 10^6 cells/mL with D-PBS.

18. Use 50 \(\mu\)L to prepare two cytospins (label a and b) and 75 \(\mu\)L to prepare two cytospins (label c and d) at 450 rpm (18.1 x \(g\)) for 6 minutes using a cytocentrifuge.

19. Airdry four slides for at least 15 min at room temperature

20. Fix slides (b) and (d) only with methanol for 10 minutes. Leave slides (a) and (c) air dried. Label the slides with the e-code and Visit number, in pencil, wrap in foil and store in a clean and dry environment in slide storage boxes at room temperature.
Figure 1: Summary of the sputum processing procedure

Process at 4°C within 2 hours of expectoration

Select sputum (if necessary using inverted microscope)

Weigh and incubate with 8 x volume D-PBS

Gently aspirate with pasteur pipette, vortex for 15 seconds

Rock on bench rocker for 15 minutes on ice

Centrifuge 790 xg for 10 mins @ 4°C

Add 4 x volume 0.2 % Sputolysin(DTT)

Vortex for 15 seconds and rock on bench rocker for 15 minutes on ice

Vortex for a further 15 seconds and filter through 48µm nylon gauze (pre-wet with D-PBS)

Perform total cell count and viability by trypan blue exclusion method in Neubauer haemocytometer

Centrifuge 790 xg 10 minutes

Resuspend cell pellet in D-PBS to 0.5 x 10^6/mL

Prepare 2 cytopspins by placing 50µl (each) – labelled (a) and (b) - and 2 cytopspins 75µL (each) – labelled (c) and (d) - cell suspension in cups of cytocentrifuge and centrifuge at 450rpm for 6 minutes

Air dry all the pre-labelled slides and fix slides (b) and (d) with methanol.

Remove 4 volumes of supernatant

Centrifuge 1500 xg for 10 mins @ 4°C

Sub aliquot and store supernatant at −70°C
2.4 Labelling Fixed Slides and Sputum Supernatant

Each slide should be pre-labelled in pencil at site, before the cytospins are made, with the following information:

- Study site number
- Subject number
- Subject initials
- Date sample was collected

The air dried slides (a) and (c) for each patient for each visit will be placed in a slide shipper box for dispatch, within 2 weeks of being processed. The methanol fixed slides (b) and (d) should be retained at the site or shipped separately to the central lab – as specified for individual study. The slide shipper boxes will be labelled using pre-printed labels with the following information:

- Requisition and account numbers
- Enrollment code
- Randomization code
- Visit number
- Study code
- Sample name (Sputum slides)

The frozen portions of sputum supernatant should be labelled using pre-printed labels with the following information:

- Requisition and account numbers
- Enrollment code
- Randomization code
- Visit number
- Study code
- Sample name (Sputum Aliquot)

The labelled tubes of supernatant will be stored in freezer bags at -70°C.
2.5. **Cell Counting Method and Recording of Data**

One slide from the 2 slides submitted for each sample will be selected for counting based on readability. As far as is reasonably possible, one person will be responsible for reading all slides prepared for this study. A proportion of slides (i.e. 10%) will be re-read by an independent trained observer to minimise the possibility of observer error.

The slides will be counted as follows:

All parts of the slide should be examined for cell counting. This can be done in either of two ways:
- start at the centre and move outwards to the top left and then in a clockwise direction to bottom right.
- start at the top left and move field by field to the right and then move down to bottom right and move field by field back to the left.

The following count will be performed on each slide:
- A 400 cell count (non-squamous cells), differentiating between eosinophils, neutrophils, macrophages, epithelial cells and lymphocytes.

The counts for each batch of slides will be recorded on a Sputum Cytology Worksheet. This form will therefore contain the results from multiple patients.

The cell counts for each slide will then be transcribed onto a copy of the Sputum Slide Results Sheet along with the following information from the Sputum Processing Worksheet: weight of sputum, total cells in sample, % squamous cell contamination, total leukocytes in sample, % viability of leukocytes. Each Sputum Slide Results Sheet will therefore contain the data from **one slide only**.
CLINICAL STUDY PROTOCOL

AZALEA Study

A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma

Version 6, 3rd April 2013

REC reference: 11/LO/0779

EudraCT reference: 2011-001093-26

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<th>Date</th>
<th>Signature</th>
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PROTOCOL OUTLINE

Full Title
A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma

Short Title/Acronym
AZALEA

AZithromycin Against pLacebo in Exacerbations of Asthma

Study Management Group
Chief Investigator (CI): Professor Sebastian L Johnston

Principal Investigators (PI):

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Professor Chris Brightling

Portsmouth Hospitals NHS Trust
Professor Anoop J Chauhan

Guy’s and St Thomas’ NHS Foundation Trust
Professor Christopher Corrigan

Nottingham University Hospitals NHS Trust
Dr Tim Harrison

The Newcastle upon Tyne Hospitals NHS Foundation Trust
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Hammersmith Hospital, Imperial College Healthcare NHS Trust
Dr Philip W Ind

St Mary’s Hospital, Imperial College Healthcare NHS Trust:
Professor Sebastian L Johnston and Dr Patrick Mallia

Heart of England NHS Foundation Trust
Dr Adel H Mansur

University Hospital of South Manchester NHS Foundation Trust
Dr. Dave Singh

NHS Greater Glasgow and Clyde
Professor Neil C Thomson and Dr Rekha Chaudhuri

Study Coordination Centre
For general queries, supply of trial documentation, and collection of data, please contact:

Study Coordinator/project manager: Zahid Sattar
Address: Respiratory Medicine Department, National Heart and Lung Institute, Imperial College London, Medical School Building, St Mary’s Campus, Paddington, W2 1PG
Tel: 0207 594 2594 Fax: 020 7262 8913

Sponsor
Imperial College Academic Health Science Centre is the main research Sponsor for this study. For further information regarding the sponsorship conditions, please contact the Head of Regulatory Compliance at:
AHSC Joint Research Office
Imperial College London

510A, 5th Floor Lab Block,
Charing Cross Hospital,
Fulham Palace Road, London, W6 8RF

Tel: +44(0)203 311 0206
Fax: +44(0)203 311 0203

Funder
NIHR, Efficacy and Mechanism Evaluation Programme

Number of sites: at least 10
Multi-centre

Objective
To evaluate the efficacy of azithromycin during an acute exacerbation of asthma.

Design
Multi-centre, randomised, double-blind, placebo-controlled study

Population
Adults with a history of asthma presenting within 48 hours (of initial presentation requesting medical care) with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough with reduced PEF)

Sample size
Approximately 380 patients will be enrolled, with the goal of obtaining approximately 190 clinically and microbiologically evaluable patients per treatment arm.

Efficacy Assessment Principal Criteria

Primary Outcome
Diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

Secondary Outcomes
- The following additional efficacy endpoints:
  - Health status assessed by acute asthma QoLQ (Juniper)
  - Health status assessed by Mini Asthma QoLQ (Juniper)
  - Pulmonary Function tests (FEV1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes will be assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies (the efficacy of Telithromycin was only assessed at 10 days).
- Time to 50% reduction in symptom score

**Exploratory analyses**
- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial *C. pneumoniae* and/or *M. pneumoniae* status
- Assessment of efficacy outcomes in relation to initial standard bacteriologic status
- Assessment of efficacy outcomes in relation to initial virologic status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status

**Clinical Safety Assessment**
Adverse event reporting, physical examinations, vital signs, and clinical laboratory parameters.

Additionally, a hierarchical model at 3 levels (including day at the lowest level) will be fitted to allow an assessment of trends over time in primary and secondary endpoints.

Analysis will be performed on an intention to treat (ITT) basis.

**Study duration and dates**
The study will start June 1st 2011 for development of CRFs and training of personnel. Subject recruitment is proposed to start 1st September 2011 and end in April 2014. Laboratory analyses, data cleaning, analysis and reporting will take a further 9 months.
STUDY SCHEDULE

Flow Chart of Study Procedures

<table>
<thead>
<tr>
<th>Study Procedure</th>
<th>Visit 1 Day 1 Within 48 hrs of initial presentation</th>
<th>Visit 2 Day 5</th>
<th>Visit 3 Day 10</th>
<th>Visit 4 Follow up Visit Day 42</th>
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<td>Informed consent</td>
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<td>Inclusion/Exclusion criteria review</td>
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<td>Medical/Surgical history</td>
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<tr>
<td>Record previous &amp; concomitant treatments</td>
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<td>Sputum for cell differential and mediators in supernatant**</td>
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<td></td>
<td></td>
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<tr>
<td>Full Blood Count (FBC)</td>
<td>X</td>
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<tr>
<td>Dispense diary- Diary training</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diary review</td>
<td>X  X</td>
<td></td>
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<tr>
<td>Return Diary to investigator</td>
<td>X  X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Health outcomes assessment - Acute Asthma QoLQ (Juniper)</td>
<td>X  X  X</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Health outcomes assessment - MiniAQLQ (Juniper)</td>
<td>X  X  X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Randomisation and Dispense study medication</td>
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<tr>
<td>Collect and count unused drug</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AE review</td>
<td>X  X  X  X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* if indicated

**see project specific SOP for details of sample collection
## ABBREVIATIONS AND DEFINITIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>AE</td>
<td>Adverse event</td>
</tr>
<tr>
<td>AR</td>
<td>Adverse reaction</td>
</tr>
<tr>
<td>CRF</td>
<td>Case report form</td>
</tr>
<tr>
<td>FEF₂₅₋₇₅%</td>
<td>Forced Mid-Expiratory Flow Rate</td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>Ratio of forced expiratory volume in one second to forced vital capacity</td>
</tr>
<tr>
<td>GCP</td>
<td>Good clinical practice</td>
</tr>
<tr>
<td>HIV</td>
<td>Human immunodeficiency virus</td>
</tr>
<tr>
<td>ICS</td>
<td>Inhaled corticosteroids</td>
</tr>
<tr>
<td>ITT</td>
<td>Intent-to-treat</td>
</tr>
<tr>
<td>MHRA</td>
<td>Medicines and Healthcare Products Regulatory Agency</td>
</tr>
<tr>
<td>OCS</td>
<td>Oral corticosteroids</td>
</tr>
<tr>
<td>PC</td>
<td>Predefined change</td>
</tr>
<tr>
<td>PCA</td>
<td>Predefined change abnormal</td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>PEF</td>
<td>Peak expiratory flow</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function tests</td>
</tr>
<tr>
<td>REC</td>
<td>Research ethics committee</td>
</tr>
<tr>
<td>SAE</td>
<td>Serious adverse event</td>
</tr>
<tr>
<td>SAR</td>
<td>Serious adverse reaction</td>
</tr>
<tr>
<td>SUSAR</td>
<td>Suspected unexpected serious adverse reaction</td>
</tr>
</tbody>
</table>
1. INTRODUCTION AND STUDY RATIONALE

Importance of asthma exacerbations
Asthma is the most prevalent respiratory disease, it is diagnosed in 5-10% of adults and 10-15% of children and around 30% of children report wheeze in the last year\(^1\). The major asthma morbidity and mortality are a result of acute exacerbations: 5-10% of asthmatics have been hospitalised with an exacerbation and \(~25,000\) Europeans die unnecessarily of asthma each year. Exacerbations also account for \(~50\)% of total expenditure on asthma care\(^2\). More than half of asthma patients reported having an exacerbation in the last year with \(>1/3\) children and \(>1/4\) adults requiring urgent medical care visits as a result\(^3\).

Aetiology of asthma exacerbations
Viruses and atypical bacteria: Respiratory viral infections are the major cause of asthma exacerbations in children (80-85%)\(^4,5\) and adults (75-80%)\(^6-8\). However, non-viral respiratory pathogens such as *Mycoplasma pneumoniae* (*M. pneumoniae*) and *Chlamydophila pneumoniae* (*C. pneumoniae*) have also been associated with wheezing episodes and asthma exacerbations in both adults and children\(^9-13\). Interestingly, in two of these studies virus detection rates were \(~80\)%\(^9,11\), while serologic positivity for atypical bacterial infection/reactivation can be as high as 40-60\%\(^9,13\) indicating that viral and atypical bacterial infections likely interact in increasing risk of asthma exacerbation.

Bacterial infections: There is little published evidence that standard bacterial infections are important in the aetiology of asthma exacerbations\(^14\), however, patients with asthma have increased susceptibility to respiratory bacterial infections\(^15-17\), increased carriage of pathogenic respiratory bacteria identified by culture\(^18\) and molecular techniques\(^19\) and impaired interferon responses to bacterial polysaccharides\(^20\). In addition viral infection impairs innate responses important in antibacterial immunity\(^21\) and increases bacterial adherence to bronchial epithelial cells\(^22\). There is therefore good evidence that bacterial respiratory infections are both more common and more severe in asthma, and that viral infection can increase susceptibility to bacterial infection.

A recent study of 361 children with \(>800\) stable and exacerbation airway samples collected during the first 3 years of life and analysed for standard bacteria and respiratory viruses, has confirmed that acute wheezing episodes were associated with both bacterial infection (odds-ratio 2.9, 95% CI 1.9-4.3, \(p<0.001\)) and with virus infection (odds-ratio 2.8, 95% CI [1.7, 4.4], \(p<0.01\))\(^23\). We therefore hypothesise that standard bacterial infections are likely also to be important in the aetiology of asthma exacerbations in adults, and aim to investigate this in the proposed study.

Treatment of asthma exacerbations
When asthma exacerbations occur, treatment options are limited to bronchodilators and steroids. Beyond the addition of magnesium in severe exacerbations, treatments have developed very little in the last \(~50\) years. Current therapeutic strategies are of limited efficacy and development of new approaches addressing the aetiologic agents causing the exacerbations is urgently needed. Current asthma guidelines recommend specifically that antibiotic therapy should NOT be administered routinely in asthma exacerbations\(^24\).

New approaches to treatment of asthma exacerbations
If atypical bacteria are causal or contributory factors in asthma exacerbation, then treatment with antibiotics with activity against mycoplasma and chlamydia species would be expected to be beneficial in asthma exacerbations. Adults with acute exacerbations of asthma and treated with Telithromycin (a ketolide antibiotic closely related to macrolides: both classes being highly active against *M. and C. pneumoniae*) as a supplement to standard care, showed a statistically significantly greater reduction in asthma symptoms (\(P<0.005\)), improvement in lung function (\(P=0.001\)) and faster recovery (\(P=0.03\)) when compared to those treated with placebo\(^13\). The magnitude of the treatment effect was also highly clinically significant, with the improvement in symptoms resulting from Telithromycin treatment being \(~50\)% greater than that observed with standard therapy (plus placebo), improvement in lung function being almost 100% greater, and importantly, recovery time to a 50%
improvement in clinical symptoms 3 days faster in those receiving active treatment. This treatment therefore had a clear therapeutic effect; however this study requires confirmation in a second similar study, before revision of guidelines could be considered. Ideally confirmation would be with a further study with Telithromycin, however issues with toxicity have limited use of Telithromycin to severe life threatening infections.

The macrolide antibiotic Azithromycin is a safe and well tolerated alternative that has been used for many years in the treatment of respiratory disease, but has thus far not been studied in acute exacerbations of asthma. We therefore hypothesise that treatment with Azithromycin might be of benefit in treatment of acute asthma exacerbations. This current study will therefore investigate the effectiveness of Azithromycin as a supplement to standard care for adult patients with acute exacerbations of asthma, following as closely as possible the design of the Telithromycin study, with the aim of providing confirmation or otherwise of those results.

**Need for this study**

There are no systematic reviews or, and no published reports of clinical trials investigating efficacy of Azithromycin in the treatment of asthma exacerbations. There are no similar studies registered on Clinicaltrials.gov. The only somewhat similar study is NCT00266851 which plans to enrol 200 adult patients with asthma, either stable persistent or in exacerbation and treat for 3 months, to answer the question: will a 12-week treatment with the antibiotic, Azithromycin, result in a statistically significant and clinically meaningful improvement in overall asthma symptoms and other patient-oriented asthma outcomes one year after initiation of treatment of adult primary care patients with asthma? Thus the aims, design, timing of outcome analysis and treatment length are clearly very different from the proposed study.

**Mechanisms of activity of macrolide/ketolide antibiotics in treatment of asthma exacerbations**

Macrolide/ketolide antibiotics might have therapeutic benefit in treating asthma exacerbations through treatment of either standard or atypical bacteria or both. In addition, both macrolide and ketolide antibiotics have anti-inflammatory properties that are independent of their antibacterial activity which may be beneficial in reducing airway inflammation, which is known to be important in the pathogenesis of asthma exacerbations\(^7,25\). In addition to these three possible mechanisms of action, we also believe antiviral activity is a 4th possible mechanism.

We have previously reported that impaired type I and type III interferon production by virus infected bronchial epithelial cells and macrophages is important in the pathogenesis of asthma exacerbations\(^20,26\). We have also recently shown that Azithromycin, but not Erythromycin or Telithromycin, significantly increased rhinovirus induced type I and type III interferon and interferon-stimulated anti-viral protein production in primary bronchial epithelial cells, as well as significantly reducing rhinovirus replication and release in bronchial epithelial cells\(^27\). Azithromycin has also been shown to reduce illness severity in a mouse model of viral bronchiolitis\(^28\). Thus Azithromycin has potential to have direct anti-viral activity by augmenting production of those interferons we have already shown to be deficient in asthma exacerbations\(^25,26\), and this property may make it a better treatment option than Telithromycin, which does not appear to have this property\(^27\). A further mechanistic aim of our study therefore, is to investigate frequencies of standard bacterial, atypical bacterial and viral infections in these exacerbations to determine the relative importance of each of these infections, and of possible co-infections with one or more agents, in the aetiology of acute exacerbations of asthma in adult subjects. We will also perform subgroup analyses to determine whether any treatment benefit observed is greater in those with evidence of one or more of these infections, with the aim of shedding some light on the possible mechanism(s) of action of Azithromycin in this context.

**Concerns re antimicrobial resistance**

This clinical trial will be important as there are significant concerns regarding development of resistance against macrolide antibiotics. Although these concerns are somewhat mitigated by the short course of therapy being studied (relative for example to ongoing clinical trials investigating long term treatment in severe asthma), determining whether Azithromycin has efficacy in this context will, if
the study is negative help limit inappropriate use of antibiotics (in a recent study of adult asthma exacerbations, 57% of subjects received antibiotics\textsuperscript{29}).

If the study is positive, determining the frequencies of detection of standard bacterial, atypical bacterial and viral infections in these exacerbations, combined with the subgroup analyses assessing efficacy of the intervention in those with evidence of one or more of these infections should help guide use of such therapies in subgroups of asthma exacerbations that may respond better to such therapies, as well as guiding future investigation of efficacy of alternative antibiotics with shorter durations of action or different spectra of antimicrobial/viral/inflammatory activity.

**Choice of and duration of therapy**

Although the course of therapy is only 3 days, Azithromycin has a multiple-dose tissue half-life of 68hrs and will therefore persist in the lung at significant concentrations for around 10 days after initiation of a 3 day course of therapy\textsuperscript{30}. The main aim of this study is to determine whether the Telithromycin results can be validated in a study with a similar antibiotic, with a similar mechanism and duration of action. Telithromycin was given for 10 days (the standard licensed duration of therapy for other respiratory indications) and the primary outcome variable was assessed at 10 days\textsuperscript{13}. Since our aim is to determine whether the Telithromycin results can be validated, we feel it is important to use the same primary outcome variable and as similar a duration of action as is possible (given that we cannot use Telithromycin due to liver toxicity). This was one reason why we chose to study Azithromycin rather than other macrolide antibiotics. Other reasons for choosing Azithromycin are its antiviral activity that are not shared with other macrolides\textsuperscript{27}, a more favourable drug interaction profile\textsuperscript{30} and excellent concentration at sites of infection\textsuperscript{30}.

If the study is positive, we will wish to conduct further studies of antibiotics (macrolide and non-macrolide) with shorter durations of action to determine if these are also effective.

**Sputum and serum biomarkers of exacerbation aetiology can be used to direct therapy and improve clinical outcomes in COPD exacerbations**

The Chief Investigator (CI) has recently collaborated with Prof Brightling on a study investigating whether sputum and serum biomarkers of exacerbation aetiology can be used to direct therapy in COPD exacerbations. This study followed patients with COPD for 1 year at stable and exacerbation visits. A large panel of biomarkers were measured in sputum and blood and viruses, atypical bacteria and standard bacteria were assessed in sputum by PCR and routine diagnostic bacterial culture. Biomarkers that differentiated exacerbations associated with bacteria, viruses or eosinophilic airway inflammation were investigated. 145 patients (101 men, 44 women) entered the study and 182 exacerbations were captured from 86 patients. 55%, 26% and 28% met definitions for bacteria, virus or sputum eosinophil associated exacerbations. Respectively each of the associated exacerbations were best identified by sputum IL-1β (area under receiver operator curve 0.89 (95% confidence interval 0.83 to 0.95), serum CXCL10 (IP-10) 0.83 (0.70 to 0.96), and percentage peripheral blood eosinophils 0.85 (0.78 to 0.93).

In a follow on study, these subjects were randomized to be treated directed by biomarker analysis (patients with blood eosinophilia received steroids, those without did not), or in the non biomarker group all patients received steroid treatment, to determine whether biomarker directed therapy could improve treatment outcomes. In this study all patients received antibiotics. This follow on study found that biomarker directed therapy significantly reduced steroid usage and improved symptom recovery in the biomarker directed group, compared to the non-directed group (Bafadel M, Johnston SL, Brightling CE et al. NEJM [revised and re-submitted]). This study therefore clearly validates the concept that biomarkers of COPD exacerbation phenotypes can be identified and further, that they can be used in directing therapy to improve clinical outcomes in COPD exacerbations. Serum IP-10 has already been associated with virus induced asthma exacerbations in adults\textsuperscript{31}, however, very few other potential biomarkers have been investigated in this context, and none have been investigated for exacerbations associated with bacterial infections. For this study we propose to collect and store the samples that would permit the biomarker analyses to be carried out at a later date, and if the clinical outcomes of the study are positive, we hope to take forward further projects to
carry out the biomarker analysis. Whether these biomarkers can be applied to direct therapy and improve clinical outcomes will warrant further investigation in future studies in asthma exacerbations.

**Are patterns of airway inflammation associated with aetiology and treatment outcomes?**

Different patterns of airway inflammation have been identified in both stable asthma and during exacerbations – these have been classified as neutrophilic, eosinophilic, mixed granulocytic or pauci-granulocytic. However it is not known whether these different patterns of inflammation are associated with different aetiologies for the exacerbation, nor whether they are related to treatment outcome. We therefore finally aim to characterise the inflammatory cell profiles in sputum at presentation, to determine whether exacerbation aetiology as well as any possible treatment benefit are related to the types of airway inflammation present (neutrophilic, eosinophilic, mixed or pauci-granulocytic).
2. STUDY OBJECTIVES

2.1 Primary Objective

Efficacy will be assessed using diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

2.2 Secondary Objectives

The secondary objectives of the study are to look at:

- The following additional efficacy endpoints:
  - Health status assessed by acute asthma QoLQ (Juniper)
  - Health status assessed by Mini Asthma QoLQ (Juniper)
  - Pulmonary Function tests (FEV1, FVC, FEV1/FVC ratio, PEF, FEF25-75%, FEF50%)
- Primary and secondary outcomes will be assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies
- Time to 50% reduction in symptom score

Exploratory analyses

- Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days
- Assessment of efficacy outcomes in relation to initial C. pneumoniae and/or M. pneumoniae status
- Assessment of efficacy outcomes in relation to initial standard bacteriologic status
- Assessment of efficacy outcomes in relation to initial virologic status
- Assessment of efficacy outcomes in relation to initial sputum inflammatory cell status
3. **STUDY DESIGN**

This is a multi-centre, randomised, double-blind, placebo-controlled study

The duration of therapy with study medication (active and/or placebo) will be 3 days, with post-therapy up to 10 days and a follow-up visit at six weeks.

The following diagram summarises the design for the study:
4. SELECTION OF PATIENTS

4.1 Number of patients
As calculated in Section 10.3, page 35, approximately 380 patients should be enrolled and treated with study medication at the study centres. The expected enrollment is approximately 38 patients per site. Enrollment into the randomisation phase of the study will be stopped when the anticipated or actual subject numbers have been achieved across all study sites.

4.2 Pre-Randomisation Evaluations
Patients eligible for the study will be approached by a member of the research team within 48 hours of the patient presenting to medical care with an acute exacerbation of asthma. The patient will be given the study patient information leaflet to read and have the study explained to them. They will be given time to consider their participation and discuss the project and told that participation is voluntary. When a patient has had sufficient time to consider participation and they have agreed to take part they will be asked to sign a consent form. All screening procedures are described below in Section 7.

4.3 Inclusion criteria
Patients meeting all of the following criteria will be considered for admission to the study:

- Adults, either sex, ages 18-55 years or age 56 to 65 with < 20 pack year smoking history or >65 with <5 pack year smoking history
- Patients with a documented history of asthma for >6 consecutive months, and
- Patients presenting within 48 hours (of initial presentation to medical care) with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough and/or reduced PEF) and requiring a course of oral steroids
- Patients with a PEF or FEV1 less than 80% of predicted normal or patient’s best at presentation, at recruitment or in the time elapsed between presentation and recruitment
- Patients must be able to complete diaries and quality of life questionnaires.
- Patients must sign and date an informed consent prior to any study procedures.

4.3 Exclusion criteria
Patients presenting with any of the following will not be included in the study:

- Patients with known prolongation of the QT interval, a history of torsades de pointes, congenital long QT syndrome, bradyarrhythmias or uncompensated heart failure, patients on drugs known to prolong the QT interval and patients with ongoing proarrhythmic conditions such as uncorrected hypokalemia or hypomagnesemia, clinically significant bradycardia, and patients receiving Class IA (quinidine, procainamide) or Class III (dofetilide, aminodarone, sotalol) antiarrhythmic agents.
- Smokers aged 56-65 with a >20 pack year history, or aged >65 with ≥5 pack year history
- Patients requiring immediate placement in ICU
- Patients who used oral or systemic antibiotics within 28 days prior to enrolment
- Patients with known impaired hepatic function (ALT/AST > 2 ULN)
- Patients with significant lung disease (including COPD) other than asthma
- Patients with > 20mg oral corticosteroid maintenance therapy
- Patients requiring other antibiotic therapy
- Patients who are receiving other medications or who have other disease conditions or infections that could interfere with the evaluation of drug efficacy or safety
• Women who are breast-feeding or are pregnant, as demonstrated by a urine pregnancy test carried out before exposure to study medication or the start of any study procedure that could pose a risk to the foetus
• Patients with suspected or known hypersensitivity to, or suspected serious adverse reaction to Azithromycin or any of the macrolide or ketolide class of antibiotics, erythromycin or to any excipients thereof
• Patients who have received treatment with any other investigational drug within 1 month prior to study entry, or have such treatment planned for the study period during treatment and follow up phase
• Patients with a concomitant condition (including clinically relevant cardiovascular, hepatic, neurologic, endocrine, or other major systemic disease) making implementation of the protocol or interpretation of the study results difficult
• Patients with mental conditions rendering them unable to understand the nature, scope, and possible consequences of the study.
• Patients unlikely to comply with the protocol, e.g., uncooperative attitude, inability to return for follow-up visits.
• No subject will be allowed to enrol in this study more than once.

4.4 Withdrawal of patients

An early withdrawal visit will be performed on patients who are withdrawn from the study prematurely, at any time between receiving the first dose of study medication and visit 3, day 10.

Patients may be withdrawn from the study for the following reasons:
• At their own request or at the request of their legally authorised representative
• If, in the investigator’s opinion, continuation in the study would be detrimental to the subject’s well-being

Patients must be discontinued from study medication under the following circumstances:
• Pregnancy (every attempt must be made to follow-up patients who become pregnant to determine the outcome of the pregnancy)
• Deterioration of the clinical condition or delayed response—in the investigator's opinion—at least 48 hours after beginning study treatment; e.g., two doses of study medication
• Addition of any additional oral or parenteral antibiotic during study days 1-10
• The occurrence of severe unexpected reactions to the drug on more than one occasion

In all cases, the reason for withdrawal must be recorded on the case report form and in the subject’s medical records. The subject must be followed up to establish whether the reason was an adverse event and, if so, this must be reported in accordance with the procedures in Section 8.

The investigator must make every effort to contact patients lost to follow-up.

4.5 Replacement of patients

Patients will not be replaced.
5. STUDY TREATMENTS

5.1 Details of study treatments

The following study treatments will be used in this study:

<table>
<thead>
<tr>
<th>Drug code</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zithromax</td>
<td>Containing 500 mg of azithromycin (2×250mg capsules), over-encapsulated with lactose powder</td>
</tr>
<tr>
<td>Placebo</td>
<td>Size 00 capsules containing lactose powder</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Manufacturer</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pfizer</td>
<td>Capsules, over-encapsulated by Bilcare</td>
</tr>
<tr>
<td>Bilcare</td>
<td></td>
</tr>
</tbody>
</table>

The identity of the treatment regimen will be blinded by encapsulating active medication in opaque (00) capsules to match the placebo.

5.2 Dosage schedule

All patients in the study will receive, per randomised allocation, treatment with either azithromycin or the placebo. Those randomised to azithromycin will receive 500 mg azithromycin (two 250 mg capsules) once a day for 3 days (this is the routine dose given in clinical care). Those patients randomised to the placebo will receive two placebo capsules once a day for 3 days. The duration of treatment with study medications will be 3 days. Patients will be instructed to take study medication at least 1 hour before or 2 hours after food and if they are taking antacids to take the study drug at least 1 hour before or 2 hours after the antacids.

The time of administration of the first and last doses of study medication, and the labeling on the study medication containers should be documented on the case report form. The first dose will be given in the presence of a member of the research team.

5.3 Treatment assignment

The study medication will be administered only to patients included in this study following the procedures set out in the study protocol.

All sites taking place in the study will be allocated a unique three digit site number. All patients who have signed an informed consent document will receive a three-digit subject number that will be used to identify the subject throughout the study. Subjects will be enrolled into the project using the online project InForm database and will be allocated their three-digit subject number sequentially as they are added to InForm so that no two subjects at the same site are allocated the same number. A patient can then be identified by their site number and subject number.
Randomisation will be web-based via access to a secure Imperial College server. Patient allocation will be stratified by centre performed in random length blocks.

Patients withdrawn from the study retain their subject number and their randomisation number, if already given. New patients must always be allotted a new subject number and, if applicable, a new randomisation number.

5.4 Blinding, packaging, and labeling

The identity of the study medications will be blinded and packaged according to the randomisation schedule and supplied to the investigator by Bilcare with code break envelopes. Over-encapsulated Zithromax capsules and placebo capsules will be placed into child resistant tamper evident containers and a randomised label applied to each container. When a patient is randomised into the study the details of the randomised label will be replicated in the patient’s study notes.

Emergency identification of study medication/unblinding

If it is medically imperative to know what study medication the subject is receiving, the investigator or authorised person should open the relevant code break envelope that corresponds to the randomisation label on the patients study drug container, exposing the blinded information. The investigator or the person who breaks the blind must record the date and the reasons for doing so in the case report form and in the subject’s medical records. The principal investigator must be contacted immediately to determine whether the subject should be withdrawn from the study.

The reason for unblinding should be assessed to determine if it is an adverse event and if so the reporting requirements in Section 8 should be followed.

5.5 Supplies and accountability

The pharmacist will inventory and acknowledge receipt of all shipments of study medication. All study medication must be kept in a locked area with access restricted to designated study personnel. The study medication must be stored in accordance with the manufacturer's instructions. The investigator or pharmacist will also keep accurate records of the quantities of study medication dispensed, used, and returned by each subject. The site monitor will periodically check the supplies of study medication held by the investigator or pharmacist to ensure accountability of all study medication used. At the conclusion of the study, all unused study medication and all medication containers will be returned to the hospital pharmacy for disposal unless other arrangements have been approved by the sponsor.

5.6 Compliance

The patients first return visit after being given the drug/placebo will be on day 5 at which point they should have completed the full dose of drug. Compliance will be assessed by capsule counts at this visit and unused study medication and all medication containers will be collected. Details will be recorded on the case report form.

5.7 Undesirable drug effects

Azithromycin is well tolerated with a low incidence of side effects. The following undesirable effects are noted in the summary of product characteristics:
Common effects (> 1/100, < 1/10)
- Gastrointestinal disorders – nausea, vomiting, diarrhoea, abdominal discomfort (pain/cramps)

Uncommon effects (> 1/1000, < 1/100)
- Nervous system disorders – dizziness/vertigo, somnolence, headache, convulsions, taste perversion, syncope
- Gastrointestinal disorders – Loose stools, flatulence, digestive disorders, anorexia, dyspepsia
- Skin and subcutaneous tissue disorders – allergic reactions including pruritus and rash
- Musculoskeletal, connective tissue and bone disorders – arthralgia
- Reproductive system and breast disorders – vaginitis

Rare effects (>1/10000, <1/1000)
- Blood and lymphatic system disorders – thrombocytopenia, occasional transient mild neutropenia
- Psychiatric disorders – aggressiveness, agitation, anxiety and nervousness
- Nervous system disorders – paraesthesia and asthenia, insomnia and hyperactivity
- Ear and labyrinth disorders – impaired hearing, deafness, ringing in the ears
- Cardiac disorders – palpitations, arrhythmias including ventricular tachycardia and rare reports of QT prolongation and torsades de pointes
- Vascular disorders – hypotension
- Gastrointestinal disorders – constipation, discolouration of the tongue, pancreatitis, pseudomembranous colitis
- Hepato-biliary disorders – hepatitis and cholestatic jaundice have been reported, including abnormal liver function test values, as well as rare cases of hepatic necrosis and hepatic dysfunction, which in rare instances have resulted in death
- Skin and subcutaneous tissue disorders – allergic reactions including angioneurotic oedema, urticaria and photosensitivity; serious skin reactions such as erythema multiforme, Stevens-Johnson syndrome and toxic epidermal necrolysis
- Renal and urinary disorders – interstitial nephritis and acute renal failure
- General disorders – anaphylaxis including oedema (leads in rare cases to death), candidiasis, fatigue, malaise
6. PRIOR AND CONCOMITANT ILLNESSES AND TREATMENTS

6.1 Prior and concomitant illnesses

Illnesses first occurring or detected during the study, and worsening of concomitant illness during the study, are to be regarded as adverse events and must be documented as such on the case report form (see Section 8).

6.2 Prior and concomitant treatments

All additional treatments being taken by the patients on entry to the study or at any time during the acute phase of the study are regarded as concomitant treatments and must be documented on the appropriate pages of the case report form.

Standard care treatment for asthma will be allowed.

6.2.1 Antibiotic treatments

No oral or parenteral concomitant antibiotic treatments are permitted for 28 days prior to randomisation, nor for the duration of study up to day 10. Patients receiving oral or parenteral antibiotic treatments that cannot be discontinued are not eligible for inclusion in the study, and patients requiring such antibiotic treatments other than the study medication during the treatment period and up to study day 10 must be withdrawn and the reasons noted on the case report form.

6.2.2 Nonantibiotic treatments

If concomitant nonantibiotic treatments are considered to be necessary for the subject's welfare and are unlikely to interfere with the study medication, they may be given at the discretion of the investigator.

6.2.3 Precautions

In patients taking concomitant antacids, azithromycin should be taken at least 1 hour before or 2 hours after the antacid. Patients will also be instructed to take study medication at least 1 hour before or 2 hours after food.

Interactions between macrolide antibiotics and the following drugs have been reported. If patients are receiving these medications, please monitor as per your standard clinical practice.

- theophylline
- cyclosporine
- carbamazepine
- cimetidine
• digoxin
• ergot derivatives
• methylprednisolone
• nelfinavir
• terfenadine
• zidovudine
• didanosine
• rifabutin
• coumarin-type oral anticoagulants including warfarin
7. STUDY PROCEDURES AND SCHEDULE

7.1 Overview of data collection

The primary efficacy analysis will be to demonstrate clinical efficacy of oral azithromycin (500 mg OD) treatment for 3 days in patients with acute exacerbations of asthma.

The primary assessment of clinical efficacy will be evaluated using the following criteria assessed 10 days after randomisation:

- Diary card summary symptom score

Additional efficacy variables will include:

- Health Status assessed by acute asthma QoLQ (Juniper)
- Health Status assessed by mini asthma QoLQ (Juniper)
- Pulmonary Function tests (FEV₁, FVC, FEV₁/FVC ratio, PEF, FEF₂₅₋₇₅%, FEF₅₀%)
- Time to 50% reduction in symptom score
- Primary and secondary outcomes to be assessed at days 5 and 10 post randomisation
- Trends in primary and secondary outcomes over the time course of the exacerbation up to 14 days
  - Assessment of the patient’s clinical improvement relative to initial
    - C. pneumoniae and/or M. pneumoniae status
    - standard bacteriologic status
    - virologic status
    - sputum inflammatory cell status

The assessment of safety will be performed using the following criteria:

- Adverse events

Additional assessment will include:

- Compliance with study medication, assessed by counting unused capsules at the end-of-therapy visit (visit 2, day 5)

7.2 Description of study days

Study visits can be carried out either at the participating site or at the participant’s home if this is requested.

A summary of the Study Schedule is shown on page 8 above. The following observations will be made during the study according to the schedules below:

Visit 1 Day 1 (Pre-therapy/Entry visit – within 48 hours of initial presentation)
Location of visit: Participating site or participant’s home

Inclusion/exclusion criteria

Conduct of study explained to patient and a signed informed consent obtained prior to the performance of any study procedures

- Demographics
- Medical/surgical history (per signed patient history or previous medical records) including documentation of:
  - asthma diagnosis
  - tobacco consumption
- Recording of time since presentation to medical care
- Assessment of severity of asthma at presentation using BTS guidelines on asthma severity
- Previous and concomitant treatment including previous antibiotic treatment
- Pulmonary function tests including FEV\textsubscript{1}, FVC, FEV\textsubscript{1}/FVC ratio, \textit{FEF}_{25-75\%}, \textit{FEF}_{50\%} and peak flow (See Section 7.3.1 Pulmonary Function Tests)
- Urine pregnancy tests for women of childbearing potential
- Serum for biomarkers and serology for atypical pathogens. (See project specific SOP for Instructions for collecting samples).
- Nasal and throat swab and nasal mucus for PCR for all common respiratory viruses
- Spontaneous or induced sputum for PCR for all common respiratory viruses and atypical bacterial pathogens. (See project specific SOP for details of sample collection).
- Spontaneous or induced sputum for cytospins for cell differential and processing for sputum supernatant for mediators and biomarkers. (See project specific SOP for details of sample collection).
- Quantitative culture of sputum for standard bacteria
- Blood sample for full blood count
- Dispense patient diary and provide instructions for completing the diary (See 7.3.1 Patient’s Daily Recordings)
- Health outcomes assessment – Acute Asthma QoLQ (Juniper)
- Health outcomes assessment – MiniAQLQ (Juniper)
- Randomisation and study medication dispensed
- Instructions to bring all unused study medication to the next visit for verification
- Adverse event reporting requirements
- Appointment for next visit

Visit 2 Day 5 (End-of-therapy visit) – this visit can be varied +/- one day either side of day 5 and exceptionally by +/- two days

Location of visit: Participating site or participant’s home
• Previous and concomitant treatment
• Pulmonary function tests FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅₋₇₅%, FEF₅₀%, peak flow (See Section 7.3.1 Pulmonary Function Tests)
• Diary review
• Health outcomes assessment – Acute Asthma QoLQ (Juniper)
• Health outcomes assessment – MiniAQLQ (Juniper)
• Collect and count unused drug
• Adverse event review
• Appointment for next visit

Visit 3 Day 10 - this visit can be varied +/- one day either side of day 10 and exceptionally by +/- two days
Location of visit: Participating site or participant’s home
• Previous and concomitant treatment
• Pulmonary function tests, FEV₁, FVC, FEV₁/FVC ratio, FEF₂₅₋₇₅%, FEF₅₀%, peak flow (See Section 7.3.1 Pulmonary Function Tests)
• Diary review
• Health outcomes assessment – Acute Asthma QoLQ (Juniper)
• Health outcomes assessment – MiniAQLQ (Juniper)
• Adverse event review
• Appointment for next visit

Visit 4 Day 42 (Follow-Up Visit) - this visit can be varied +/- two weeks either side of day 42
Location of visit: Participating site or participant’s home
• Serology for atypical pathogens. (See project specific SOP for instructions for collecting samples).
• Adverse event review
7.3 Methods of data collection

7.3.1 Efficacy data

Pulmonary Function Tests (PFTs)

A spirometer that meets all ATS recommendations should be used. PFTs will be performed at Visits 1 to 3. PFTs will be measured three times in a consistent position (standing or sitting) throughout the study. The best FEV\textsubscript{1}, FVC, FEV\textsubscript{1}/FVC ratio, FEF\textsubscript{25-75%}, FEF\textsubscript{50%} and peak flow are to be recorded in the CRF as stated below:

1. Forced expiratory volume in one second (FEV\textsubscript{1}) in litres;
2. Forced vital capacity (FVC) in litres;
3. Forced expiratory volume in one second/forced vital capacity (FEV\textsubscript{1}/FVC) ratio;
4. Forced Mid-Expiratory Flow Rate (FEF\textsubscript{25-75%}) in litres/sec;
5. Forced Expiratory Flow Rate at 50% (FEF\textsubscript{50%}) in litres/sec;
6. Peak expiratory flow (PEF) in litres/min

Patient's Daily Recordings

All patients will be supplied with a diary in which to record Salbutamol use, asthma symptom ratings and number of nighttime awakenings due to asthma symptoms. At Visit 1 patients will be instructed regarding recording of information in the diary (see below) and asked to complete the diary each day for 10 days at the end of the day (with the nocturnal questions referring to the previous night). They will be reminded of the recording instructions at Visits 2 and 3.

I. Daytime symptom diary scale questions

1. How often did you experience asthma symptoms today?
   - 0 None of the time
   - 1 All of the time
   - 2
   - 3
   - 4
   - 5
   - 6

2. How much did your asthma symptoms bother you today?
   - 0 Not at all bothered
   - 1
   - 2
   - 3
   - 4
   - 5
   - 6 Severe bothered

3. How much activity could you do today?
   - 0 More than usual activity
   - 1
   - 2
   - 3
   - 4
   - 5
   - 6 Less than usual activity

4. How often did your asthma affect your activities today?
   - 0 None of the time
   - 1 All of the time
   - 2
   - 3
   - 4
   - 5
   - 6

II. Nocturnal diary scale question
1. Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning?)
   - No
   - Once
   - More than once
   - Awake “all night”

III. Number of inhalations of Salbutamol will be recorded in the diary. Each patient should be reminded that Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically.

IV. Study medication will be recorded in the diary. Any concomitant medication use will be recorded in the diary.

V. Adverse Events - patients will record all unusual health related events in the diary regardless of relationship to medication.

Clinical sample collection

Respiratory samples

- A nasal mucus sample and nasal and throat swab will be taken at Visit 1 for PCR for viruses and atypical bacteria.
- At Visit 1 in patients with a productive cough, deep expectorated sputum will be collected after rinsing the mouth with sterile water.
- In patients unable to produce an adequate sample of spontaneous sputum, sputum will be induced according to published protocols using isotonic saline if the visit is taking place at the recruiting site.

The sputum will be processed for PCR, standard bacteriology and cytospin and supernatant production. If sputum cannot be obtained at visit 1 because of nonproductive cough or for any other reason, this must be documented on the case report form.

Serology

Acute (Visit 1) and convalescent (Follow up visit day 42) serum samples will be obtained, and forwarded to the Chief Investigator’s laboratory.

(see project specific SOP for instructions for Collecting Samples, for details).

7.3.2 Safety data

Adverse events

Adverse events observed by the investigator or reported by the subject will be documented as described in Section 8

7.3.3 Health outcomes data

Health outcomes will be measured to determine:

Overall assessment of symptom resolution during the first ten days based on global subject diary assessment

Health Status at visits 1 to 3
- Acute AsthmaQoLQ (Juniper)
- Mini Asthma QOLQ (Juniper)
8. PHARMACOVIGILANCE

8.1 Definitions

8.1.1 Adverse Event (AE):

Any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. *An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational medicinal product (IMP), whether or not considered related to the IMP.*

The adverse event may be:

- A new illness
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness
- An effect of the study medication, including comparator
- A combination of two or more of these factors.

Variations in clinical observations are both common and expected consequences of the diseases of the upper respiratory tract and their resolution processes. An increase of these infection-related events may be seen in the subject population under study. They should be reported as adverse events when, in the opinion of the investigator, they deviate from normal in terms of frequency, intensity or duration. If the event meets the criteria of serious, then the event must be reported as a serious adverse event (see below).

8.1.2 Adverse Reaction (AR)

All AEs judged by either the reporting investigator or the sponsor as having reasonable causal relationship to a medicinal product qualify as adverse reactions.

8.1.3 Unexpected Adverse Reaction

An AR, the nature or severity of which is not consistent with the applicable product information (ie the summary of product characteristics (SmPC) for azithromycin). *Side effects documented in the SmPC which occur in a more severe form than anticipated are also considered to be unexpected.*

8.1.4 Serious Adverse Event (SAE) or Serious Adverse Reaction (SAR)

Any untoward medical occurrence or effect that at any dose:

- **Results in death**
- **Is life-threatening** – refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe
- **Requires hospitalisation, or prolongation of existing inpatients’ hospitalisation**
- **Results in persistent or significant disability or incapacity** – there is a substantial disruption of a person’s ability to carry out normal life functions
- **Is a congenital abnormality or birth defect**
Medical judgement should be exercised in deciding whether an AE/AR is serious in other situations. Important AE/ARs that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious.

Hospitalisation of the patient as a direct result of the asthma exacerbation should not be recorded as an SAE as this is part of the patients’ routine clinical care and not related to their participation in the trial.

### 8.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

Any suspected adverse reaction related to an IMP that is both unexpected and serious.

### 8.2 Causality

Most adverse events and adverse drug reactions that occur in this study, whether they are serious or not, will be expected treatment-related toxicities due to the drugs used in this study. The assignment of the causality should be made by the investigator responsible for the care of the participant using the definitions in the table below.

If any doubt about the causality exists the local investigator should inform the Chief Investigator. Other clinicians may be asked to advise in some cases.

In the case of discrepant views on causality between the investigator and others, all parties will discuss the case. In the event that no agreement is made, the MHRA will be informed of both points of view.

<table>
<thead>
<tr>
<th>Relationship</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated</td>
<td>There is no evidence of any causal relationship</td>
</tr>
<tr>
<td>Unlikely</td>
<td>There is little evidence to suggest there is a causal relationship (e.g. the event did not occur within a reasonable time after administration of the trial medication). There is another reasonable explanation for the event (e.g. the participant’s clinical condition, other concomitant treatment).</td>
</tr>
<tr>
<td>Possible</td>
<td>There is some evidence to suggest a causal relationship (e.g. because the event occurs within a reasonable time after administration of the trial medication). However, the influence of other factors may have contributed to the event (e.g. the participant’s clinical condition, other concomitant treatments).</td>
</tr>
<tr>
<td>Probable</td>
<td>There is evidence to suggest a causal relationship and the influence of other factors is unlikely.</td>
</tr>
<tr>
<td>Definitely</td>
<td>There is clear evidence to suggest a causal relationship and other possible contributing factors can be ruled out.</td>
</tr>
<tr>
<td>Not assessable</td>
<td>There is insufficient or incomplete evidence to make a clinical judgement of the causal relationship.</td>
</tr>
</tbody>
</table>

### 8.3 Period of observation

For the purposes of this study, the period of observation extends from the time the subject gives informed consent until 7 days after the last dose of study medication.
8.4 Reporting Procedures

All adverse events should be reported. Depending on the nature of the event the reporting procedures below should be followed. Any questions concerning adverse event reporting should be directed to the sponsor in the first instance. A flowchart is given below to aid in the reporting procedures.

All adverse events that occur after the subject has signed the informed consent must be documented on the pages provided in the case report form. The initial report must be as complete as possible, including details of the current illness and (serious) adverse event, and an assessment of the causal relationship between the event and the study medication.

All patients who have adverse events, whether considered associated with the use of the study medication or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed up according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the investigator considers it medically justifiable to terminate follow-up.

8.4.1 Non serious AR/AEs

All such toxicities, whether expected or not, should be recorded in the toxicity section of the relevant case report form. These should be reported to the MHRA and REC on the annual safety report form to be completed on the anniversary of the date a favourable opinion for the study was given.

8.4.2 Serious AR/AEs/SUSARs

Fatal or life threatening SAEs and SUSARs should be reported to the Chief Investigator (who will report to the sponsor) on the day that the local site is aware of the event. The SAE form asks for nature of event, date of onset, severity, corrective therapies given, outcome and causality (i.e. unrelated, unlikely, possible, probably, definitely). Additional information should be sent to the CI and sponsor within 5 days if the reaction has not resolved at the time of reporting.

SAEs
An SAE form should be completed and faxed to the Chief Investigator for all SAEs within 24 hours. The Chief Investigator should inform the sponsor of all SAEs within 24 hours of receiving notice of them. All SAEs should be recorded on the annual safety reports that are sent to the MHRA and REC on the anniversary of the date a favourable opinion for the study was given.

SUSARs
In the case of serious, unexpected and related adverse events, the staff at the site should:

Contact the Chief Investigator by phone and then complete the SAE case report form & send it immediately (within 24 hours, preferably by fax), signed and dated to the Chief Investigator together with relevant treatment forms and anonymised copies of all relevant investigations.

The Chief Investigator will notify the MHRA, the main REC and the sponsor of all SUSARs occurring during the study according to the following timelines; fatal and life-threatening within 7 days of notification and serious but non-life threatening within 15 days. All investigators will be informed of all SUSARs occurring throughout the study.
Local Principal Investigators should report any SUSARs and/or SAEs as required by their Local Research Ethics Committee and/or Research & Development Office in addition to reporting them to the Chief Investigator.

Contact details for reporting SAEs and SUSARs
Fax: 0207 262 8913, attention Professor Sebastian Johnston
Please send SAE forms to: Professor Sebastian Johnston
Tel: 0207 594 3764 (Mon to Fri 09.00 – 17.00)
9. STATISTICAL PROCEDURES

9.1 Study populations

The intent-to-treat (ITT) population is to be used for statistical analyses:

9.1.1 Eligibility for clinical efficacy analysis

The following criteria will be applied:

Population of patients eligible for the ITT analysis:
- All randomised patients, as treated, who received at least one dose of study medication and with signs and symptoms of exacerbations of asthma.

9.1.2 Eligibility for bacteriological efficacy analysis

All ITT patients who are tested for *C. pneumoniae* and/or *M. pneumoniae* status, standard bacteriologic status, virologic status or sputum inflammatory cell/mediator status at baseline will be included for the bacteriological analyses.

9.1.3 Eligibility for safety analysis

All patients, as treated, who received at least one dose of the study treatment with post baseline safety assessment will be eligible for the safety analysis.

9.1.4 Major protocol violations

Patients who fall into any of the following categories will be classed as patients with major protocol violations:
- Wrong entry diagnosis
- Previously enrolled in the study
- Missing appropriate post-treatment information
- Received more than 24 hours of treatment with other antibiotics within 28 days prior to enrollment into the study (see Section 4.3)
- Patients who are randomised into the trial and do not meet the inclusion/exclusion criteria
- Use of non study systemic antimicrobials between entry and day 10
- Insufficient treatment duration (to be defined in the analysis plan prior to unblinding). Any subject treated for at least 48 hours and judged by the investigator to be a clinical failure will be considered clinically evaluable.

Major protocol violations not limited to the above categories will be detailed in the final analysis plan prior to the unblinding of the database.

Deviations from clinical trial protocols and GCP occur commonly in clinical trials. The majority of these instances are technical deviations that do not result in harm to the trial subjects or significantly affect the scientific value of the reported results of the trial. These cases should be documented e.g. in
the case report form for the trial or trial master file, in order for appropriate corrective and preventative actions to be taken. In addition, these deviations should be included and considered when the clinical study report is produced.

Any serious breach of GCP or of the protocol is defined as one:

“which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial (this should be relevant to trial subjects in the UK); or

(b) the scientific value of the trial.”

It is the responsibility of the Sponsor to assess the impact of the breach on the scientific value of the trial. If it is decided that the breach is serious then it is the responsibility of the Principal Investigators to report it within 24 hours to the Chief Investigator and for the Chief Investigator to report within 24 hours to the sponsor. The Chief Investigator, in conjunction with the sponsor, should then submit a report of the breach to the MHRA within 7 days of becoming aware of the breach. This should be followed up by a full report when all details of the breach are known.

9.2 Statistical methods

A full Statistical Analysis Plan (SAP) will be developed and agreed by the Trial Steering Committee.

9.2.1 Analysis of efficacy data

The primary efficacy endpoint will be analysed using a 2-level hierarchical model (centre and patient as the two levels). The model will include treatment, study centre, the baseline value of the primary endpoint, and any predefined covariates. The treatment group means and the between group differences adjusted for any pre-specified covariates will be estimated using this model and the model will be used to test whether Azithromycin differs from placebo. The same hierarchical modelling will be used for all secondary endpoints.

Additionally, a hierarchical model at 3 levels (including day at the lowest level) will be fitted to allow an assessment of trends over time in primary and secondary endpoints.

9.2.2 Handling of missing data

A missing data strategy will outlined in the SAP using multiple imputation to acknowledge the associated uncertainty.

The covariates include severity of asthma; severity of exacerbation as measured by % predicted FEV and % predicted PEF at recruitment; age; gender; acute AQLQ and mini AQLQ (Juniper) at recruitment; smoking status; atypical bacterial positive status (serology or culture or PCR positives); standard bacterial status (sputum positive or negative); virus PCR positive status; seasonality; exploratory and sensitivity analyses will examine the effects of these predictors and their possible interactions with treatment effect.

The predefined limited number of subset analyses will be specified in the detailed Statistical Analysis Plan.

9.2.3 Analysis of safety data

Adverse events
Frequencies of patients with treatment-emergent adverse events, regardless of relationship to study treatment and sorted by body system, will be summarised by treatment group. Frequencies of patients with possible treatment-related adverse events will also be displayed. Frequencies of patients with adverse events including those leading to death or permanent discontinuation of study medication will be likewise summarised. Frequency tables of adverse events, displayed by intensity, will also be provided.

**Exploratory analyses**

Regression models will be used to analyse each of the exploratory questions. These will be analogous to those described above for the primary and secondary outcomes.

### 9.3 Sample size justification

The sample size calculations are based on the primary outcome: Change from baseline in diary card summary asthma symptom scores at 10 days after randomisation. Our previous study\(^\text{13}\) found the mean decrease in symptom score of 1.3 in treatment group, and 1 in the control group, resulting in the difference of -0.3 (SD 0.783) between the groups at 10 days.

Using a two-sided t-test at 1% significance level with 80% power we would need 161 patients in each group to be able to detect the same difference in asthma scores between the groups. The significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable, as well as to provide greater power for the subgroup exploratory analyses, as those subgroup analyses that were performed were uninformative in the 280 patient Telicast study\(^\text{13}\).

Taking into account the drop-out rate of 15% in the study\(^\text{13}\), we propose to recruit 190 patients in each arm of the study. To be able to run the trial within the project timelines, we intend to involve 10 centres.
10. ETHICAL AND LEGAL ASPECTS

10.1 Good Clinical Practice (GCP)

“Good clinical practice is a set of internationally recognised ethical and scientific quality requirements which must be observed for designing, conducting, recording and reporting clinical trials that involve the participation of human subjects.” - EU Directive 2001/20/EC, article 1, clause 2. Compliance with this good practice provides assurance that the rights, safety and well-being of trial subjects are protected, and that the results of the clinical trials are credible and accurate.

The procedures set out in this study protocol, pertaining to the conduct, evaluation, and documentation of this study, are designed to ensure that the investigator abides by the principles of good clinical practice (GCP) as described in articles 2 to 5 in the EU Directive 2005/28/EC as well as in the ICH Harmonised Tripartite Guidelines Topic E 6: “Guideline for Good Clinical Practice.” Compliance with these regulations also constitutes compliance with the ethical principles described in the current revision of the Declaration of Helsinki (October 2008). The study will also be carried out in keeping with local legal and regulatory requirements.

10.2 Delegation of investigator responsibilities

The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their trial-related duties and functions.

The investigator should maintain a list of sub-investigators and other appropriately qualified persons to whom he or she has delegated significant trial-related duties.

10.3 Subject information and informed consent

Before being admitted to the clinical study, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her.

An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. The document must be in a language understandable to the subject and must specify who informed the subject. Consent to enter the study must be sought from each participant only after a full explanation has been given, an information leaflet offered and time allowed for consideration. Consent can be taken by any Clinician or Clinical Research Nurse working on the project.

After reading the informed consent document, the subject must give consent in writing if they agree to participate in the study. The subject's consent must be confirmed at the time of consent by the personally dated signature of the subject and by the personally dated signature of the person conducting the informed consent discussions. If the subject is unable to read, oral presentation and explanation of the written informed consent form and information to be supplied to patients must take place in the presence of an impartial witness. Consent must be confirmed at the time of consent orally and by the personally dated signature of the subject or by a local legally recognised alternative
(e.g., the subject's thumbprint or mark). The witness and the person conducting the informed consent discussions must also sign and personally date the consent document.

A copy of the signed consent document must be given to the subject. The original signed consent document will be retained by the investigator and a copy placed in the patient’s medical records.

The investigator will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

The investigator will inform the subject’s General Practitioner (GP) about the subject’s participation in the trial if the subject has a GP and if the subject agrees to their GP being informed.

Subjects are free to withdraw from the study at any time, without giving a reason. This would not affect the standard care they receive.

10.4 Trial Governance

This trial will be sponsored by Imperial College London and the Imperial Clinical Trials Unit (ICTU) will be responsible for the coordination. ICTU provides experienced staff within an infrastructure supporting the management, monitoring and reporting of clinical trials involving investigational medicinal products (IMP’s). ICTU has systems in place to ensure compliance with the Medicines for Human Use (Clinical Trials) Regulations 2004.

Adverse Events will all be notified to the Data Monitoring and Ethical Committee as well as being reviewed by the Trial Management Committee who will respond accordingly.

This study will not open to recruitment until appropriate approvals and authorisations have been obtained from an independent ethical committee and the Medicines & Healthcare Products Regulatory Agency. Recruitment will not commence at an individual participating site until local NHS Management approval has been obtained and all local documentation is in place and all requirements have been fulfilled according to ICTU Standard Operating Procedures (SOPs).

The plans for the Governance of this trial include the involvement of the ICTU Quality Assurance (QA) Manager. The QA Manager will oversee the creation of all essential documentation to be used in this trial, which will be written with reference to the ICTU SOPs to ensure they comply with all ethical and regulatory requirements and are fit for purpose. The QA manager will also conduct a Risk Assessment on the study to ensure the development of an appropriate monitoring plan, which takes into account the particular requirements of this study. Monitoring will be implemented accordingly under the supervision of the Trial Manager. Oversight of the trial will be conducted on a day-to-day basis by the QA Manager and the Director of Operations.

Trial Documentation

ICTU carries out all its trials in compliance with its SOPs. These cover all aspects of setting up and managing multi-centre clinical trials. For example, they include templates for writing protocols, consent documents, DMEC charters and reports. They also include checklists to ensure activities eg the release of IMPs to the investigator sites, are conducted appropriately. Essential documents will also be created, collected and housed in accordance with ICH (E6) GCP Guidelines section 8.2 and regulatory requirements. A trial master file will be established at ICTU as directed by the sponsor and corresponding site files will be established at each investigator site. The trial shall not begin before all relevant documentation has been collected, distributed and verified by the monitor(s)/ trial management at or before site initiation as necessary. Similarly, trial close-out shall not be performed
before the monitors(s)/ trial management has reviewed investigator and ICTU files and verified that all necessary documents are in the appropriate files. Essential documents will be available for inspection by auditors, regulatory inspectors and study monitors.

Retention of Documents

ICTU has in place well established protocols for the protection of data and for retention of documents. Data will be stored for a minimum of 15 years (or according to changes in regulatory requirements) following completion of this trial. Data generated by this work will be processed in accordance with the Data Protection Act 1998. ICTU will adhere to the Imperial College Code of Practice, drawn up in association with the Imperial’s Data Protection Policy, relating to the collection, holding and disclosure of data relating to individuals. The Principal and Co-applicants will act as custodians of the data, and be responsible for its security.

The PI at each investigational site is responsible for the archiving of all the essential trial documents, including the Investigator Site File, in accordance with regulatory requirements. The PI will ensure the continued storage of the documents, even if s/he were to leave the clinic/practice or retire before the end of the required storage period. Delegation of these responsibilities will be documented in writing.

Trial Management

The Trial Steering Committee will have an independent chair and will also include a patient representative and the lead investigator as well as additional committee members from the project team. A Data Monitoring & Ethical Committee will also be established, and options for suitable independent candidates to chair this committee are also being explored. The Trial Management Committee will be chaired by Professor Sebastian Johnston and will be made up of the Principal Investigators (PI’s), PI’s, the Study Manager, a study Statistician and representative from ICTU.

10.5 Confidentiality

Only the subject number and subject initials will be recorded in the case report form, and if the subject name appears on any other document (e.g., pathologist report) it will be completely anonymised.

The investigator will maintain a personal subject identification list (subject numbers with the corresponding subject names) to enable records to be identified.

10.6 Approval of study protocol amendments

The REC, MHRA, R&D office and sponsor must be informed of all subsequent protocol amendments. Amendments must be evaluated to determine whether or not they are substantial and therefore if formal approval must be sought and whether the informed consent document should also be revised.

The investigator must keep a record of all communication with the REC and, if applicable, between study investigators and the REC. This also applies to any communication between the investigator (or coordinating investigator, if applicable) and the authorities.
10.7 Monitoring and audit

The Principal Investigators will permit trial related monitoring, audits, ethics review and regulatory inspections, providing direct access to source data/documents as required. The project will be monitored by the project monitor and subject to the audit and monitoring requirements of Imperial College, London and all NHS sites at which the project is taking place.

10.8 Publication Policy

All publications and presentations relating to the study must be authorised by the Chief Investigator.
11 DOCUMENTATION AND USE OF STUDY FINDINGS

11.1 Documentation of study findings

All protocol-required information collected during the study must be entered by the investigator, or designated representative, in the electronic case report form using the InForm database. Training will be provided to all project staff on the use and completion of the InForm database and a database user guide will be available. The investigator, or designated representative, should complete the case report form pages as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

A source data location list will be prepared and updated during the study. This list will be filed in both the trial master file and the investigator study file.
12. STUDY DURATION AND DATES

The study will start June 1st 2011 for development of CRFs and training of personnel. Subject recruitment is proposed to start 1st September 2011 and end in April 2014. Laboratory analyses, data cleaning, analysis and reporting will take a further 9 months.

12.1 Definition of the end of the trial

The date of the last visit of the last patient undergoing the trial.
13. FINANCES

Participants will be eligible to receive a maximum payment of £50 at visit 4 if they have attended all study visits and completed and returned all 10 symptom diaries. The payment is equivalent to the sum of £10 for attending each study visit plus £10 for returning all 10 symptom diaries. Investigators will reimburse participants directly from the AZALEA sponsor payment.
14. REFERENCES


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APPENDIX A  INSTRUCTIONS FOR ADMINISTRATION OF THE AQLQ

If possible, the AQLQ should be the first questionnaire completed during a clinic visit and should precede any discussion with a health professional. If patients discuss their health state before completing the questionnaire, the response of the health professional to the patient’s experiences may influence how the patient answers the questionnaire.

General Instructions for Questionnaire Administration

It is important to ensure that patients understand the questionnaires, so they can provide quality information throughout the study. The Acute Asthma Quality of Life Questionnaire (Acute AQLQ) is designed the find out how the patient has been feeling during the last half hour and the Mini Asthma Quality of Life Questionnaire (MiniAQLQ) has been designed to find out how the patient has been feeling during the last 3 days.

Both the Acute AQLQ and the MiniAQLQ should be interviewer administered. Study coordinators should avoid interpreting questions for patients, and encourage them to interpret questions as best they can and then answer accordingly.

It is very important that none of the questionnaires are administered to patients prior to signing the consent form, just as with any other clinical data you are collecting. Most patients will have little or no experience of completing questionnaires of this sort, so the study coordinator’s explanations and enthusiasm are important.

Interviewer Administered Questionnaires

Staff who will be administering the questionnaires should read the ‘Background, Administration and Analysis’ for all AQLQs before administering any questionnaires. The key points to remember are:

- Ask the questions exactly as worded
- Never help a patient choose a response
- Emphasise that there are no right or wrong answers
- Be neutral to the patient’s responses
- Ask all questions in the order specified
- Avoid missing data – it is vital that all questions have been answered

Answers to Patient Questions

Occasionally, a patient will express concern about his or her ability to follow these directions. There are several standard concerns patients may express:

“Should I say how I feel now, or how I’ve felt since the last visit?”

Patients should be told to respond for the period as indicated for each questionnaire ie for the last half hour for the Acute AQLQ and the last 3 days for the MiniAQLQ. If they report that they feel differently today compared to the last 3 days, ask them to consider how they have felt “on average” or “most of the time” over the previous 3 days.

“That question doesn’t apply to me.”

Occasionally, a patient will remark that a particular item on the questionnaire seems directed at someone else. For example, they may not engage in certain activities such as walking up
stairs. Ask them to put down their best guess based on what they think would happen if they did try to engage in that activity. A patient’s perception that an item “does not apply” is not a valid reason for missing data. It’s far better for them to estimate than leave it blank. Confidentiality is a concern of some patients. Whenever a questionnaire is handed to the patient, the study coordinator should reassure the patient that “all information is strictly confidential and does not become a part of your general medical record. The physician will not review this information to make treatment decisions”. Tell concerned patients of specific steps taken to protect their privacy such as referring to their records by a study identification number, not a name, and the reporting of results for groups, not individual patients. If this is confusing, tell them this research is like reporting an average grade for the class, rather than posting each student’s grade.

Problems of understanding questions should be minimal but if a patient states they do not understand the administrator should never attempt to reword or paraphrase the question. The administrator should initially repeat the question then if this does not help the correct response is “Whatever it means to you” emphasising there are no right or wrong answers.

**Specific Instructions for AQLQ Administration**
The Asthma Quality of Life instrument asks patients about symptoms, emotions, activities and environmental factors related to asthma. All of the response scales have seven options that range from no effect to a constant effect on their lives.
Sample Acute AQLQ

This questionnaire has been tested using the wording and format that follows. It is important that interviewers adhere to the exact wording when addressing patients (regular type) and follow the instructions (italics). Deviation from both wording and instructions may impair the validity of the questionnaire.

This questionnaire has been designed to find out how you have been feeling during the last half hour (30 minutes). You will be asked about your symptoms and how your asthma has made you feel.

Show the red and yellow cards to the patient and explain the scales.

Make sure that he/she has the correct card for each question and that he/she reads it before responding.

Remind the patient that you only want to know about how he/she has been during the last half hour.

Record the patient's answers on the response sheet.

I want you to tell me how much you have been bothered by your asthma during the last half hour.

1. How bothered have you been by shortness of breath during the last half hour? (Red Card)

2. How bothered have you been by coughing during the last half hour? (Red Card)

3. How much of the time have you felt afraid of not having your asthma medications available during the last half hour? (Yellow Card)

4. How much of the time have you experienced a feeling of fighting for air during the last half hour? (Yellow Card)

5. How much of the time have you felt frustrated as a result of your asthma during the last half hour? (Yellow Card)
6. How much of the time have you felt concerned about the need to use medications for your asthma during the last half hour? (Yellow Card)

7. How bothered have you been by chest tightness or chest heaviness during the last half hour? (Red Card)

8. How bothered have you been by wheezing during the last half hour? (Red Card)

9. How bothered have you been by difficulty breathing out during the last half hour? (Red Card)

10. How much of the time have you felt afraid of getting out of breath during the last half hour? (Yellow Card)

11. How much of the time have you felt concerned about having asthma during the last half hour? (Yellow Card)

RESPONSE SHEET

1. shortness of breath
2. coughing
3. afraid of not having your asthma medications available
4. fighting for air
5. frustrated
6. concerned about the need to use medications for your asthma
7. chest tightness or chest heaviness
8. wheezing
9. difficulty breathing out
10. afraid of getting out of breath
11. concerned about having asthma

RESPONSES

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</table>
RESPONSE OPTION CARDS

RED CARD

1. Extremely Bothered
2. Very Bothered
3. Quite Bothered
4. Somewhat Bothered
5. Bothered a bit
6. Hardly Bothered at all
7. Not bothered

YELLOW CARD

1. All of the time
2. Most of the time
3. Quite often
4. Some of the time
5. Once in a while
6. Hardly any of the time
7. None of the time
**Sample MiniAQLQ**

**MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE**

<table>
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<th>DATE</th>
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</table>

Please complete all questions by circling the number that best describes how you have been during the last 2 weeks as a result of your asthma.

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

<table>
<thead>
<tr>
<th>Question</th>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>Hardly Any of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Feel SHORT OF BREATH as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>2. Feel bothered by or have to avoid DUST in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>3. Feel FRUSTRA TED as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>4. Feel bothered by COUGHING?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>5. Feel AFRAID OF NOT HAVING YOUR ASThma MEDICATION AVAILABLE?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>6. Experience a feeling of CHEST TIGHTNESS or CHEST HEAVINESS?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>7. Feel bothered by or have to avoid CIGARETTES in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>8. Have DIFFICULTY GETTING A GOOD NIGHT'S SLEEP as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>9. Feel CONCERNED ABOUT HAVING ASTHMA?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>10. Experience a WHEEZE in your chest?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
<td>7</td>
</tr>
</tbody>
</table>
MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE

SELF ADMINISTERED

DATE __________

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APPENDIX B  PATIENT DIARY

All patients will be supplied with a diary in which to record Salbutamol use, asthma symptom ratings and number of nighttime awakenings due to asthma symptoms. At Visit 1 patients will be instructed regarding recording of information in the diary (see below), and asked to complete the diary each day for 10 days at the end of the day (with the nocturnal questions referring to the previous night). They will be reminded of the recording instructions at Visits 2 and 3.

I   Daytime symptom diary scale questions

1.   How often did you experience asthma symptoms today?

   0  1  2  3  4  5  6
   None of                      All of
   the time                     the time

2.   How much did your asthma symptoms bother you today?

   0  1  2  3  4  5  6
   Not at all                   Severely
   bothered                    bothered

3.   How much activity could you do today?

   0  1  2  3  4  5  6
   More than usual activity    Less than usual activity

4.   How often did your asthma affect your activities today?

   0  1  2  3  4  5  6
   None of                      All of
   the time                     the time

II  Nocturnal diary scale question

   1   Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning?)
   □ No  □ Once  □ More than once  □ Wake “all night”

III Number of inhalations of Salbutamol will be recorded in the diary. Each patient should be reminded that Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically.

IV   Study medication will be recorded in the diary. Any concomitant medication use will be recorded in the diary.

V   Adverse Events - patients will record all unusual health related events in the diary regardless of relationship to medication.
Summary of protocol changes
The following amendments were made to the trial protocol following approval of the first version of the document by the REC and MHRA:

**Version 2:**
- Addition of the name of the project manager appointed to this study
- Addition of a throat swab in case sufficient sample is not obtained from the nasal mucus and nasal swab
- Refinement of inclusion criteria to include FEV1 as well as PEF as a measurement of lung function
- Refinement of exclusion criteria to clarify the type of antibiotic use that will be excluded
- Our drug supplier (Bilcare) advised us that the placebo capsules and over-encapsulation of the Zithromax capsules will only use Lactose Powder and not Magnesium Stearate as they had originally specified. This is because they ‘are producing a small amount of capsules that will be made on the hand frame so the Magnesium Stearate powder will not be required’. The protocol was changed accordingly.
- Clarification that all standard care for asthma will be permitted
- Addition of the option of home visits for study visits 2, 3 and 4 if requested and the patient is unable to attend hospital
- Clarification of the leeway allowable for the day of each visit (to allow for weekends and participant unavailability)
- Clarification as to when the patients should complete the symptom diaries
- Inclusion of a statement that hospitalisation as a direct result of the asthma exacerbation is not an SAE as this is part of their routine clinical care and not related to their participation in the trial
- Removal of Appendix C and D (instructions for sample collection and analysis) as this was part of the study specific SOPs that will be regularly reviewed and updated.

**Version 3:**
- Refinement of inclusion criteria to include patients aged over 65 years with less than 5 pack year smoking history
- Addition of the telephone number of the project manager appointed to this study

**Version 4:**
- Refinement of the eligibility criteria to include patients presenting within 48 hours (of initial presentation to medical care) with an acute deterioration of asthma control (instead of 24 hours as in the previous protocol version)
- To allow for Visit 1 to be conducted at the recruiting site or participant’s home
- Recruitment extension to April 2014
- Minor administrative changes

**Version 5:**
- Protocol amendment to introduce participant reimbursements for completing study visits and returning all symptom diaries - participants will be eligible to receive a maximum payment of £50 at visit 4 if they have attended all study visits and completed and returned all 10 symptom diaries. The payment is equivalent to the sum of £10 for attending each study visit (1, 2, 3 and 4) plus £10 for returning all 10 symptom diaries.

**Version 6:**
- Addition of an extra exclusion criteria to reflect guidelines released from the FDA on the use of azithromycin
AZithromycin Against pLacebo in Exacerbations of Asthma

A Randomised, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy of Oral Azithromycin (500 Mg OD) as a Supplement to Standard Care for Adult Patients with Acute Exacerbations of Asthma

Chief Investigator: Professor Sebastian L Johnston

EudraCT NUMBER: 2011-001093-26
SPONSOR: Imperial College Academic Health Science Centre
FUNDER: NIHR, Efficacy and Mechanism Evaluation Programme
STUDY COORDINATION CENTRE: Imperial Clinical Trials Unit
SAP Version: Version 0.3
Date: 10th July, 2014

STATISTICAL ANALYSIS PLAN
draft

Non-confidential

Prepared by Alexina Mason (Trial Statistician until July 2014)

This is a SAP for a full report to the TSC
not all the analysis is intended for publication
Approvals

This SAP is approved by:

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<td>Chief Investigator</td>
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<td>Professor Deborah Ashby</td>
<td>Senior Statistician</td>
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Also requires TSC approval.

Version Control

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1 Background

1.1 Introduction

Asthma is the most prevalent respiratory disease, and the major asthma morbidity and mortality are a result of acute exacerbations. Respiratory viral infections are the major cause of asthma exacerbations. Additionally, patients with asthma have increased susceptibility to respiratory bacterial infections. Bacteria is increased in the lower respiratory tract in stable asthma, and is hypothesised to play a role in increasing exacerbation severity as well as stable asthma severity.

When asthma exacerbations occur, treatment options are limited to bronchodilators and steroids. Current asthma guidelines recommend specifically that antibiotic therapy should NOT be administered routinely in asthma exacerbations.

If atypical bacteria are causal or contributory factors in asthma exacerbation, then treatment with antibiotics with activity against mycoplasma and chlamydia species would be expected to be beneficial in asthma exacerbations. A double-blind, randomised, placebo-controlled study found evidence of a beneficial therapeutic effect when adult patients with acute exacerbations of asthma were treated with Telithromycin (Johnston et al., 2006). However this study requires confirmation in a second similar study, before revision of guidelines could be considered. Ideally confirmation would be with a further study with Telithromycin, however issues with toxicity have limited use of Telithromycin to severe life threatening infections.

The macrolide antibiotic Azithromycin is a safe and well tolerated alternative that has been used for many years in the treatment of respiratory disease, but has thus far not been studied in acute exacerbations of asthma. We therefore hypothesise that treatment with Azithromycin might be of benefit in treatment of acute asthma exacerbations. This study will therefore investigate the effectiveness of Azithromycin as a supplement to standard care for adult patients with acute exacerbations of asthma, following as closely as possible the design of the Telithromycin study, with the aim of providing confirmation or otherwise of those results.

1.2 Study objectives

1.2.1 Primary objectives

The primary objective is to evaluate the efficacy of azithromycin during an acute exacerbation of asthma, using the diary card summary symptom score 10 days after randomisation.

1.2.2 Secondary objectives

The secondary objectives of the study are to look at:

- additional efficacy endpoints: health status assessed by acute asthma quality of life questionnaire (QoLQ), mini asthma QoLQ, and pulmonary function tests (see Section 1.6 for further detail);
- primary and secondary outcomes assessed 5 and 10 days post randomisation to permit better estimation of optimum timing of primary/secondary outcome variables in future similar studies;
• time to 50% reduction in symptom score.
In addition, the following exploratory analyses are planned.
• Trends in primary and secondary outcomes over the time course of the exacerbation up to 10 days.
• Assessment of efficacy outcomes in relation to:
  • initial Chlamydia pneumoniae and/or Mycoplasma pneumoniae status;
  • initial standard bacteriologic status;
  • initial virologic status;
  • initial sputum inflammatory cell status.

1.3 Study population

Adults with a history of asthma presenting within 48 hours (of initial presentation requesting medical care) with an acute deterioration in asthma control (increased wheeze, dyspnea and/or cough with reduced PEF).

1.4 Study design

This is a multi-centre, randomised, double-blind, placebo-controlled study. The two treatment arms are:
1. azithromycin;
2. placebo.

1.5 Primary outcome

The primary outcome will be the diary card summary symptom score, with symptoms including wheezing, breathlessness and coughing assessed at 10 days after randomisation.

1.6 Secondary outcomes

Secondary outcomes will include the following additional efficacy endpoints:
• Health status assessed by Acute Asthma QoLQ (Juniper)
• Health status assessed by Mini Asthma QoLQ (Juniper)
• Pulmonary function tests (FEV1, FVC, FEV1/FVC ratio, FEF25–75%, FEF50%, PEF)

  FEV1: forced expiratory volume in one second in litres;
  FVC: forced vital capacity in litres;
  FEV1/FVC ratio: forced expiratory volume in one second/forced vital capacity;
  FEF25–75%: forced mid-expiratory flow rate in litres/sec;
  FEF50%: forced expiratory flow rate at 50% in litres/sec;
  PEF: peak expiratory flow in litres/min.
1.7 Sample size

The sample size calculations are based on the primary outcome: change from baseline in diary card summary asthma symptom scores at 10 days after randomisation, as used in the previous study with Telithromycin (TELICAST). The TELICAST study (Johnston et al., 2006) found the mean decrease in symptom score of 1.3 in treatment group, and 1.0 in the control group, resulting in the difference of -0.3 between the groups at 10 days.

Using a two-sided t-test at 1% significance level with 80% power, 161 patients are needed in each group to be able to detect the same difference in asthma scores between the groups assuming a standard deviation of 0.783 for both groups. The significance level of 1% in the above calculation was chosen to provide greater certainty in assessment of the primary outcome variable, as well as to provide greater power for the subgroup exploratory analyses, as those subgroup analyses that were performed were uninformative in the 280 patient TELICAST study.

Allowing for a drop-out rate of 15%, as occurred in the TELICAST study, the original proposal was to recruit 190 patients in each arm of the study. It was envisaged that to be able to run the trial within the project timelines, the involvement of ten centres would be required.

These sample size calculations were subsequently revisited during the preparation of the funding extension request to the Efficacy and Mechanism Evaluation (EME) board. At this stage recruitment had been slower than expected and the rate of missing day 10 diary cards higher than anticipated. The revised target of 175 patients per treatment arm with approximately 20% day 10 diary card missingness will have almost 90% power to detect a difference of 0.3 points (SD=0.783 in both groups) in mean symptom score between the study arms at a 5% significance level.

1.8 Randomisation

Randomisation is web-based via access to a secure Imperial College server. Patient allocation is stratified by centre performed in random length blocks.

The randomisation lists were generated by Shahrul Mt-Isa (ICTU statistician) on 30 August 2011. Details such as the block size are kept confidential and are held separately by the trials unit.

1.9 Schedule of time and events

The study started 1 June 2011 for development of CRFs and training of personnel. Subject recruitment started 17 October 2011 and is scheduled to end in April 2014. Laboratory analyses, data cleaning, analysis and reporting will take place during the subsequent nine months.

The first visit takes place within 48 hours of initial presentation to medical care with an acute deterioration in asthma control. This visit includes pre-randomisation evaluations, randomisation and dispensing of the study drug. The duration of therapy with the study medication is 3 days. There are two post therapy visits at days 5 and 10, and a follow-up visit at six weeks.

Data should be collected from each subject at all four visits: the randomisation visit (visit 1) and three follow-up visits (visits 2-4). The timing of these visits and the associated data collection
schedule are shown in Table 1.

<table>
<thead>
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<th>Visit 3</th>
<th>Visit 4</th>
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</thead>
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<tr>
<td>Day 1</td>
<td>Day 5</td>
<td>Day 10</td>
<td>Day 42</td>
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<td>(±1 day*)</td>
<td>(±1 day*)</td>
<td>(±14 days)</td>
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Table 1: Data collection schedule

Demographics
Medical/Surgical history
Nose and throat swab and nasal mucus in tissue for PCR
Spontaneous/induced sputum for PCR
Culture of sputum for standard bacteria (quantitative)
Sputum for cell differential and mediators in supernatant
Full Blood Count (FBC)
Previous and concomitant treatment
Pulmonary function tests
Diary review
Acute Asthma QoLQ (Juniper)
Mini Asthma QoLQ (Juniper)
Unused drug count
Serology for atypical pathogens
Adverse event review

* exceptionally can be varied by ±2 days

2 General Considerations

2.1 Analysis strategy

The clinical efficacy analyses will be carried out on an intention-to-treat basis. Outcomes which are recorded at multiple time-points (diary card symptom scores, quality of life questionnaires and pulmonary function tests) will be analysed using a three level hierarchical model to take account of the structure in the data (time-point within patient within centre). Further detail can be found in Sections 5.4 and 5.5.

Prior to fitting statistical models, an exploratory analysis of the baseline variables and outcome measures will be completed. This will include producing summary tables (examples are shown in Appendix A), and exploring the ranges and distributions of the baseline variables and outcome measures using graphical methods. To check that baseline variables are balanced by site, site specific tables and plots will also be generated. The validity of the underlying assumptions for the proposed models will be checked and alternatives sought if necessary. Standard checks of model fit will also be carried out, and again the model specifications will be adjusted if necessary. The reasons for any changes will be fully documented.
2.2 Definition of datasets for analysis

2.2.1 Safety analysis

This will include all patients who received at least one dose of study medication, allocated to treatment arm on a per protocol basis.

2.2.2 Clinical efficacy analysis

This will be based on all randomised patients who received at least one dose of study medication, allocated to treatment arm on an intention-to-treat basis.

2.3 Data management

The trial data will be collected and managed using the InForm system, which is an electronic data capture system built around an Oracle database. The InForm system includes validation rules for data entry to help ensure data accuracy, and has a full audit trail of data entry and changes. Two types of InForm queries can be raised: automatic queries triggered by the inbuilt checks and manual queries raised by the trial monitor or other data checker. Automatic checks will include checking that values are within acceptable ranges, and consistency of values for individual patients. Manual checks include flagging incomplete data and transcription errors. Both types of queries will be resolved by the data enterers, and rechecked as necessary.

A risk assessment was carried out on 18 May 2011, which classified AZALEA as a medium risk study, and full details are held by the QA Manager. A monitoring plan commensurate with this level of risk was then developed by the Trial Manager (Laura Robison) and reviewed by the Trial Sponsor (Lucy Parker) and QA Manager (Lisa Smith). An outline of this plan is held in the study Trial Master File (TMF), with further detail available from the Monitoring Manual. In accordance with this plan, the trial monitor will carry out at least 2 monitoring visits or 1-3 per annum, in addition to site initiation and close-out visits for each site. During these visits, 100% of the informed consent forms and serious adverse events will be checked. In addition, there will be source data verification of a randomly selected 50% of subjects for eligibility, existence, drug delivery (to patients), end points, adverse events, protocol deviations and concomitant medication (primarily antibiotics). The monitoring records will be filed in the TMF.

If problems or fundamental issues become apparent in the on-going checking that forms part of the statistical analysis, the trial statistician will raise these with the principal statistician who will consult with the appropriate individuals. Any such action and subsequent decisions will be documented.

2.4 Missing data

Every effort will be made to minimise missing baseline and outcome data in this trial, but some level of missing data is inevitable. As far as possible, the reasons for the missing data will be recorded either as part of a protocol deviation or using the InForm comment facilities.
Before starting the data analysis, the level and pattern of the missing data in the baseline variables and outcomes will be established by forming appropriate tables. Additionally, the likely causes of any missingness will be summarised. This information will be used to determine whether the level and type of missing data has the potential to introduce bias into the analysis results for the proposed statistical methods, or substantially reduce the precision of estimates related to treatment effects. If necessary, these issues will be dealt with using multiple imputation (Sterne et al., 2009; Kenward and Carpenter, 2007) or Bayesian methods for missing data (Daniels and Hogan, 2008) as appropriate.

3 Outcomes and Variables of Interest

3.1 Data recorded on InForm

Details about the data captured using InForm can be found in the AZALEA annotated study book. These data include:
- randomisation details;
- baseline evaluation;
- study drug and concomitant treatment information;
- safety data;
- protocol deviations;
- patient symptom diary scores;
- answers to the acute asthma and mini asthma questionnaires;
- pulmonary function test results;
- details about the collection of biological samples, and the results of their analysis.

3.2 Biological samples

Biological samples are collected as described below, and the results transferred from spreadsheet to InForm following analysis at the end of the study. The virology and bacteriology will be carried out by the lab technician at St. Marys, and the sputum cell counts will be analysed in Leicester.

3.2.1 Blood sample

A blood sample is taken at visit 1 for a full blood count.

3.2.2 Respiratory samples

The following are taken at visit 1 for PCR for viruses and atypical bacteria:
- nasal mucus sample;
- nasal swab;
- throat swab.
If possible, a sputum sample will also be taken at visit 1, and will be processed for PCR, standard bacteriology and cytospin and supernatant production.

3.2.3 Serology for atypical pathogens

Serum samples are obtained at visits 1 and 4.

3.3 Diary card

The patient diary card is shown in Appendix C. The daily diary includes four questions measuring daytime asthma symptoms, in which patients rated the frequency and severity of their symptoms on a 7-point scale (with 0 denoting no symptoms and 6 denoting severe symptoms). The summary diary symptom score will be calculated as the mean of these four daytime activity scores (the frequency of asthma symptoms, the severity of asthma symptoms, the level of activity, and the effect of asthma on activity) recorded at the end of the day.

The scores from the four individual questions are entered into InForm, and the mean score will be calculated from these as part of the statistical analysis. Item missingness is not expected. If any item missingness does occur, then if two or three of the individual questions have been answered, the score(s) for the missing question(s) will be interpolated from the previous and subsequent day scores where possible. Otherwise the summary diary score for that day will be treated as missing.

3.3.1 Definition of day 1 for patient diary cards

For AZALEA, day 1 is defined as the day of randomisation and start of the study medication, and in general the first diary card should correspond to this day. However, if the patient started their medication late in the day, they may fill in their first diary card the day after randomisation. The time of randomisation is not recorded on InForm, but can be obtained using the emails sent by InForm to the AZALEA account as proof of randomisation when a patient is automatically or manually randomised, and will be provided by the trial manager on a spreadsheet.

Using this information, the day 1 diary card of each patient will be defined to be the first diary that is completed within 24 hours of randomisation. If no diary card has been completed within this time-frame, their day 1 diary card will be treated as missing. Diary cards for days 2-10 will be determined in a similar way.

3.4 Acute asthma and mini asthma questionnaires

The acute asthma and mini asthma quality of life questionnaires are shown in Appendix C. Both questionnaires consist of a number of questions, and each patient should answer both questionnaires on three occasions. InForm automatically calculates mean scores from the individual question scores, provided that answers to all the questions have been recorded.

If patients miss visits 1, 2 or 3, then the associated questionnaires will be missing, and a small amount of additional questionnaire missingness is expected. Item missingness is not expected, but
should it occur the overall score and associated domain score will be treated as missing, as the instructions for using these questionnaires indicate that any missing items will invalidate the score.

### 3.4.1 Acute AQLQ

The acute asthma questionnaire asks patients how they have been feeling during the previous half hour. It consists of 11 questions, each scored on the scale 1-7, where 1 represents no effect and 7 constant effect. The scores for each question are entered into InForm individually.

These questions can be grouped into two domains: symptoms (Questions 1, 2, 4, 7, 8 and 9) and emotional function (Questions 3, 5, 6, 10 and 11). InForm automatically calculates a mean score for each domain, and a mean overall score.

- Acute AQLQ overall score
- Acute AQLQ mean response - Symptoms
- Acute AQLQ mean response - Emotional function

### 3.4.2 Mini AQLQ

The mini asthma questionnaire is designed to find out how patients have been feeling during the last three days. It consists of 15 questions, and like the acute asthma questionnaire each is scored on the scale 1-7 and the scores for each question are entered into InForm individually.

The mini asthma questionnaire has four domains: symptoms (Questions 1, 4, 6, 8 and 10), activity limitations (Questions 12, 13, 14 and 15), emotional function (Questions 3, 5 and 9) and environmental stimuli (Questions 2, 7 and 11). As for the acute asthma questionnaire, InForm automatically calculates a mean score for each domain and a mean overall score.

- MiniAQLQ overall score
- MiniAQLQ mean response - Symptoms
- MiniAQLQ mean response - Activity Limitations
- MiniAQLQ mean response - Emotional Function
- MiniAQLQ mean response - Environmental Stimuli

### 3.5 Pulmonary function tests

Pulmonary functions tests (PFTs) are carried out at visits 1, 2 and 3, using a spirometer. PFTs will be measured three times and the best FEV\(_1\), FVC, FEV\(_1\)/FVC ratio, FEF\(_{25-75}\%), FEF\(_{50}\%) and PEF is recorded on the Pulmonary Function form in InForm on each occasion.

In addition to the actual measurements, this form also records predicted measurements and percentage predicted measurements. The predicted measurements are based on age, height and gender and can either be generated by an electronic spirometer or looked up in published tables. All the percentage predicted measurements are automatically calculated by InForm.
3.6 Time to 50% reduction in symptom score

Time from day 1 to 50% reduction in initial symptom score is a secondary outcome and will be calculated using the mean daytime score from the diary cards. This variable will be calculated as the number of days until the first occasion on which the score is at least 50% lower than the day 1 score (it is possible for this score to come down, but then to increase above 50% again). Patients who reach the 50% reduction will be considered to have had this event; all other patients will be treated as censored at the day of their last recorded diary score (usually day 10, but earlier for patients with missing diary card scores).

4 Interim Analysis

There will be no interim analysis.

5 Final Analysis Plan

5.1 Recruitment details

Details about patient enrolment, treatment allocation, follow-up and inclusion in analysis will be provided using a patient flow diagram as recommended by the CONSORT statement (Schulz et al., 2010). Recruitment will be summarised by centre. A breakdown of the reasons for exclusion will also be provided in tabular form.

5.1.1 Protocol deviations

Listings and summaries of the protocol deviations will be produced.

5.1.2 Compliance with study drug schedule

Any unused drug is collected during the end-of-therapy visit (visit 2, day 5), and study medication compliance assessed by counting unused capsules. Using this information, further detail will be provided on the protocol deviations associated with study drug compliance, including a table of level of compliance by treatment arm.

5.2 Baseline characteristics

Baseline characteristics of all randomised subjects will be summarised by treatment group using appropriate descriptive statistics (see for example, Appendix A, Tables A1-A4). We will also produce tables to check for any differences in baseline characteristics between centre.
5.3 Safety data analysis

Protocol reporting time frames for adverse events are from the time the subject gives informed consent until seven days after the last dose of study medication. Using the information recorded on the adverse event eCRF, each adverse event will be categorised using MedDRA coding System Organ Class (SOC) terms by a designee of the Chief Investigator. Adverse events will be summarised by the following tables:

- number of adverse events and the number of subjects who have adverse events by category and treatment group;
- number of adverse events by category and relationship to study medication;
- number of adverse events reported for individual subjects by treatment group.

In addition, the following listings will be produced:

- all adverse events classified as cardiac disorders;
- all serious adverse events.

5.4 Primary outcome analysis

5.4.1 Exploratory analysis of the primary outcome

As a data check for outliers, we will produce a series of longitudinal plots (one for each centre) of diary score for each patient, differentiating between treatment arm. For each centre and for all centres combined, we will plot the mean diary score for each treatment arm against day. We will also produce boxplots of diary score by treatment arm for each day.

Additionally, a table of summary statistics of the diary score by day and treatment arm will be produced (including mean, sd, median, lower and upper quartiles).

5.4.2 Assessment of missing data for the primary outcome

Missing data in the patient diary will potentially take one of several forms: no patient diary returned for any day (patient missingness), all data missing for one or more days (day missingness) and data missing for some but not all the individual questions for a particular day (item missingness). Of these, the level of item missingness is expected to be minimal (see Section 3.3 for a description of how this will be dealt with if it does occur). Tables will be generated to assess the level and pattern of each type of missingness. Additionally, a listing of the likely reasons for the missingness will be formed using relevant protocol deviation descriptions and other comments recorded on the eCRFs. Differences between centres will also be investigated.

Plots comparing the trajectories of the mean scores from each individual question for patients with complete data and those with incomplete data will be created to check for systematic differences. This will be repeated for the summary patient score.
5.4.3 Main analysis of the primary outcome

Modelling patient diary scores

Substantial missingness, likely to be in excess of 20%, is expected for the day 10 diary cards. However, many of the patients with missing day 10 diary card results will have diary card results from previous days. To answer the question posed by the primary objective, we plan to use information from all patients who returned at least some diary cards, not just those with day 10 diary card results. This will be done by fitting a three level hierarchical model to the diary scores collected from day 1 to day 10. Any diary card results collected outside this 10 day time-frame will be excluded from the main analysis. If there are a substantial number of diary cards collected outside this time-frame, they will be included in a subsidiary analysis.

Let $DS_{id}$ represent the diary score for patient $i$ on day $d$, $d = 1, \ldots, 10$, and $t(i)$ represent the treatment given to individual $i$ (azithromycin or placebo). Then we will model $DS_{id}$ as the sum of three components: an intercept term, a change over time term and a residual error term, i.e.

$$DS_{id} = \text{intercept}_i + \text{change over time}_{t(i)d} + \text{residual error}_{id}. \quad (1)$$

Possible choices for each of these components are outlined below. The options explored for the primary analysis will be determined by the results of the exploratory analysis, and the final choice will be the simplest model that satisfies standard checks of model fit (e.g. residual plots).

Intercept term

The intercept term will estimate the diary score on day 1 (the day of randomisation and start of the study medication). This term will comprise an individual level random effect, which will be drawn from a distribution parameterised using the associated centre level random effect. Hence the unexplained variation in the diary scores will be split into three components corresponding to the three levels of the model, i.e. the variation attributable to the centre (between centre variation) and the individual (between individual variation), as well as the residual variation (within individual variation).

Additionally, baseline covariates can be incorporated into the model at the individual level. None will be incorporated for the initial analysis unless the baseline characteristics analysis reveals a substantial imbalance. Further analyses will examine the effect of incorporating baseline variables (age, gender, asthma severity, smoking history and asthma exacerbation).

Change over time (cot) term

This term will capture the change in the diary score from the start of the study medication (day 1), hence time will enter the model as $day - 1$. The simplest assumption would be a linear change over the period, however alternatives may need to be considered as the rate of change may not be constant over the 10 day period. Alternatives are to include a quadratic term or use splines. The coefficients in this term will be dependent upon treatment.

Residual error term

We will start by assuming that the residual errors have a Normal distribution. An alternative is to assume that these errors follow a heavier tailed distribution such as a t distribution with 4 degree of freedom, which will provide robustness to outliers.
Mathematical detail for the proposed model is provided in Appendix B.1.

This model will produce unbiased results if the assumption that the missing diary scores are missing at random (MAR) is true. Robustness to departures from this assumption will be investigated using sensitivity analysis, determined by the missing data assessment described in Section 5.4.2.

**Reporting of primary outcome analysis**

The reporting described in this section assumes that the models have been fitted using a Bayesian framework. (If a classical framework is used, point estimates and confidence intervals will be presented rather than posterior distributions for Q1. Q2 will no longer be applicable.)

The primary objective, to evaluate the efficacy of azithromycin 10 days after randomisation, will be addressed by answering the question

Q1 What is the difference in the change in diary score from day 1 (baseline) to day 10 between azithromycin and placebo?

This will be done using the proposed model by calculating the quantity, day10diff, as

\[
\textit{day10diff} = \text {cot term at day 10 for azithromycin} - \text {cot term at day 10 for placebo}
\]

Using a Bayesian framework the entire posterior distribution of day10diff will be presented in addition to appropriate summary measures.

We will also directly answer the question

Q2 What is the probability that the change in diary score from day 1 (baseline) to day 10 is 0.3 points greater for azithromycin compared to placebo?

Santanello et al. (1999) reported that the average minimal patient perceivable improvement for this symptom score was -0.31 points, so 0.3 can be considered a clinically significant difference.

Similarly, differences to days 2-9 will also be presented as a further analysis of the trend over the 10 day period. We chose day 10 for the initial analysis because it was the primary outcome in TELICAST, but we will also present differences for days 2-9 as part of the primary analysis because the greatest difference between treatment arms may be seen earlier.

**5.5 Secondary outcome analysis**

For all secondary outcomes, an exploratory analysis and assessment of missing data will be completed prior to their main analysis. This will be analogous to that outlined for the primary outcome in Sections 5.4.1 and 5.4.2.

**5.5.1 Acute asthma and mini asthma questionnaires analysis**

Hierarchical models, similar to that specified for the primary outcome, will be used to analyse the acute asthma and mini asthma questionnaires.
5.5.2 Pulmonary function test analysis

Again, hierarchical models similar to that specified for the primary outcome will be used to analyse the pulmonary function tests. Missing data for these tests are expected to be due to the spirometer not recording some measures. As this is unrelated to the patient outcome, it is reasonable to assume that this missingness will be uninformative and that multi-level models fitted to all observed data will provide unbiased parameter estimates.

5.5.3 Time to 50% reduction in symptom score analysis

Kaplan-Meier curves of time to 50% reduction in symptom score will be produced for each treatment arm (truncated at 10 days).

6 Sub-studies

These will comprise the following subgroup analyses:
1. Eosinophilic, neutrophilic, mixed and neither;
2. Bacteria culture positive or negative in sputum;
3. Viral testing positive or negative in nasal swab, throat swab or sputum;
4. Atypical bacteria positive or negative in nasal swab, throat swab, sputum or serological testing.
A Tables

This section contains examples of the tables we plan to produce as part of the statistical analysis.

Table A1: Baseline characteristics of patients by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Azithromycin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age (years): median(lq,uq)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>female</td>
<td></td>
<td></td>
</tr>
<tr>
<td>male</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Asthma severity</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal asthma severity over the last 6 months†: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>step 1: mild intermittent asthma</td>
<td></td>
<td></td>
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<tr>
<td>step 2: regular preventer therapy</td>
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<td></td>
</tr>
<tr>
<td>step 3: initial add-on therapy</td>
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<td></td>
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<tr>
<td>step 4: persistent poor control</td>
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<td></td>
</tr>
<tr>
<td>step 5: continuous or frequent use of oral steroids</td>
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<td></td>
</tr>
<tr>
<td><strong>Smoking history</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoking status: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>current smoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>former smoker</td>
<td></td>
<td></td>
</tr>
<tr>
<td>never smoked</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pack years: median(lq,uq)*</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Asthma exacerbation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severity at initial presentation‡: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>near-fatal asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>life threatening asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>acute severe asthma</td>
<td></td>
<td></td>
</tr>
<tr>
<td>moderate asthma exacerbation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mild asthma exacerbation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time from presentation to study drug§ (hours): median(lq,uq)*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* lq=lower quartile; uq=upper quartile
† step 1 = patient taking inhaled short-acting \( \beta_2 \) agonist as required
step 2 = patient taking inhaled steroids at 200-800 mcg/day (BDP or equivalent)
step 3 = in addition to step 2 the patient is taking long-acting \( \beta_2 \) agonist (LABA), leukotriene receptor antagonist or SR theophylline
step 4 = in addition to step 3 the patient has increased use of inhaled steroids at > 800 and ≤ 2000 mcg/day (BDP or equivalent) or taking an additional drug eg leukotriene receptor antagonist or SR theophylline
step 5 = Patient using steroid tablet daily and inhaled steroids at 2000 mcg/day (BDP or equivalent), or other treatments for severe asthma
‡ severity of the asthma exacerbation at the time of initial presentation to medical care
§ time interval between initial presentation to medical care and administration of the study drug
<table>
<thead>
<tr>
<th></th>
<th>Azithromycin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with sputum sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptococcus pneumoniae: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemophilus influenzae: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxella catarrhalis: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlamydia pneumoniae and Mycoplasma pneumoniae*: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae-positive and M. pneumoniae-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae-negative and M. pneumoniae-positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae-positive and M. pneumoniae-negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C. pneumoniae-negative and M. pneumoniae-negative</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* C. pneumoniae = Chlamydia pneumoniae; M. pneumoniae = Mycoplasma pneumoniae
Table A3: Sputum virology results of patients by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Azithromycin</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of patients with sputum sample</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhinovirus: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Other Picornaviruses: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adenoviruses: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bocavirus: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
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<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory Syncytial Virus: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Influenza AH1/AH3/B: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Parainfluenza viruses 1-3: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
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<td></td>
</tr>
<tr>
<td>HMPV: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
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<tr>
<td>negative</td>
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<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coronaviruses 229E and/or OC43: n(%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>result not available</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table A4: Pulmonary function measurements of patients at baseline (visit 1) by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Azithromycin (n=)</th>
<th>Placebo (n=)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>mean (sd)</td>
</tr>
<tr>
<td>Actual measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (litres)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (litres)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₂₅₋₇₅% (litres/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₅₀% (litres/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF (litres/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (litres)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (litres)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₂₅₋₇₅% (litres/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₅₀% (litres/sec)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF (litres/min)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Percentage predicted measurements</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₂₅₋₇₅%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEF₅₀%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEF</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* lq=lower quartile; uq=upper quartile
† (maximum, minimum)
B Statistical Detail

B.1 Models for patient diary scores

A three level hierarchical model for analysing the diary card scores was described in Section 5.4.3. Here we provide mathematical detail for its specification within a Bayesian framework. Equivalent models can be specified within a more classical framework.

Let $DS_{id}$ represent the diary score for patient $i$ on day $d$, $d = 1, \ldots, 10$, $t(i)$ (or $t_i$) represent the treatment given to individual $i$ (azithromycin or placebo) and $c(i)$ represent the centre at which individual $i$ was treated. Then Figure 1 provides a graphical representation of a three level hierarchical model, including individual level random effects drawn using centre level random effects.

Figure 1: Graphical representation of diary card score model

The random effect and other unknown parameters have the following interpretation:

- $\alpha_i$: diary card score on day 1 for individual $i$ (individual level random effect)
- $\gamma_{c(i)}$: mean diary card score on day 1 across all individuals treated at centre $c(i)$ (centre level random effect)
- $\delta$: overall mean diary card score on day 1 across all centres and individuals (mean hyperparameter of the centre level random effects)
\( \sigma^2_{[c]} \): between centre variance in the diary card scores \\
\( \sigma^2_{[i]} \): between individual variance in the diary card scores \\
\( \sigma^2_{[e]} \): residual variance in the diary card scores (reflects measurement error and within individual variance after accounting for change over time) \\
\( \boldsymbol{\beta} \): treatment dependent parameters associated with the change in score over time \\

A hierarchically centered parameterisation has been chosen as this generally leads to better mixing of MCMC chains. The variance of the individual random effects is assumed to be constant across the centres for simplicity, but this can be allowed to vary by centre to improve model fit if necessary.

Assuming a linear relationship between diary card score and time, then this hierarchical model can be defined as follows:

\[
\begin{align*}
DS_{id} & \sim \text{Normal}(\mu_{id}, \sigma^2_{[e]}) \\
\mu_{id} & = \alpha_i + \beta_t(i) \times (d - 1) \\
\alpha_i & \sim \text{Normal}(\gamma_{c(i)}, \sigma^2_{[i]}) \\
\gamma_{c(i)} & \sim \text{Normal}(\delta, \sigma^2_{[c]})
\end{align*}
\]  

(B1)

where \( \beta_t(i), \delta, \sigma^2_{[c]}, \sigma^2_{[i]}, \) and \( \sigma^2_{[e]} \) are given vague prior distributions. The \( \beta_t(i) \times (d - 1) \) term can be elaborated to allow for a more complex relationship between diary card score and time as discussed under the ‘change over time term’ heading in Section 5.4.3. Baseline covariate adjustment can be incorporated by adding individual level baseline covariates terms into the calculation of \( \mu_{id} \).

Using the model specified by Equation B1, we would calculate \( 9(\beta_A - \beta_P) \) to answer Q1 (Section 5.4.3), where \( A \) represents azithromycin and \( P \) represents placebo. Q2 can be answered by calculating the probability directly using the \textit{step} function in the BUGS software.
C  Diary Card and Asthma Quality of Life Questionnaires

This appendix contains:

- the patient diary;
- the acute asthma quality of life questionnaire;
- the mini asthma quality of life questionnaire.
AZALEA PATIENT DIARY

Please complete a diary card every day up to and including day 10 after you joined the AZALEA study. Complete this at the end of the day (with the nocturnal questions referring to the previous night).

Keep the completed diaries and return to your AZALEA Research Nurse at your next appointment.

Subject Number:  (to be completed by Research Nurse)

Date completed:  /  /     (to be completed by AZALEA participant)

Time:    :        am/pm (delete as appropriate)

SECTION I: Daytime symptom diary scale questions (please circle the appropriate number)

1. How often did you experience asthma symptoms today?
   0 1 2 3 4 5 6
   None of the time  All of the time

2. How much did your asthma symptoms bother you today?
   0 1 2 3 4 5 6
   Not at all bothered  Severely bothered

3. How much activity could you do today?
   0 1 2 3 4 5 6
   More than usual activity  Less than usual activity

4. How often did your asthma affect your activities today?
   0 1 2 3 4 5 6
   None of the time  All of the time

SECTION II: Nocturnal diary scale question (please tick the appropriate box)

Did you wake up with asthma symptoms? (This can be awakening in the middle of the night or on awakening in the morning)
   ☐ No       ☐ Once       ☐ More than once       ☐ Awake “all night”

SECTION III: Salbutamol use

Number of inhalations of Salbutamol today and during the previous night? ________
(Please note Salbutamol should be used only as needed for symptoms, not on a regular basis or prophylactically).
AZALEA PATIENT DIARY

Subject Number: 

SECTION IV: AZALEA study medication (please tick the appropriate box)

Did you take the AZALEA study medication today?

Yes ☐   No ☐ If No please state the reason:

Please use the space below to record any other medication you have taken today.

SECTION V:

Please record any other health related events which you have experienced today regardless of whether you think it is related to the study medication.

If you feel any of these events should be addressed urgently please contact your AZALEA research team as soon as possible.

Thank you for taking time to complete this diary card.
ACUTE ASTHMA QUALITY OF LIFE QUESTIONNAIRE

INTERVIEWER-ADMINISTERED

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© The Acute Asthma Quality of Life Questionnaire is copyrighted. It may not be altered, sold (paper or electronic), translated or adapted for another medium without the permission of Elizabeth Juniper.

OCTOBER 2000
This questionnaire has been tested using the wording and format that follows. It is important that interviewers adhere to the exact wording when addressing patients (regular type) and follow the instructions (italics). Deviation from both wording and instructions may impair the validity of the questionnaire.

This questionnaire has been designed to find out how you have been feeling during the last half hour (30 minutes). You will be asked about your symptoms and how your asthma has made you feel.

Show the red and yellow cards to the patient and explain the scales.

Make sure that he/she has the correct card for each question and that he/she reads it before responding.

Remind the patient that you only want to know about how he/she has been during the last half hour.

Record the patient's answers on the response sheet.

1. How bothered have you been by shortness of breath during the last half hour? (Red Card)
2. How bothered have you been by coughing during the last half hour? (Red Card)
3. How much of the time have you felt afraid of not having your asthma medications available during the last half hour? (Yellow Card)
4. How much of the time have you experienced a feeling of fighting for air during the last half hour? (Yellow Card)
5. How much of the time have you felt frustrated as a result of your asthma during the last half hour? (Yellow Card)
ACUTE ASTHMA QUALITY OF LIFE QUESTIONNAIRE

INTERVIEWER-ADMINISTERED

PATIENT ID________________________

DATE______________________________

Page 2 of 3

6. How much of the time have you felt concerned about the need to use medications for your asthma during the last half hour? (Yellow Card)

7. How bothered have you been by chest tightness or chest heaviness during the last half hour? (Red Card)

8. How bothered have you been by wheezing during the last half hour? (Red Card)

9. How bothered have you been by difficulty breathing out during the last half hour? (Red Card)

10. How much of the time have you felt afraid of getting out of breath during the last half hour? (Yellow Card)

11. How much of the time have you felt concerned about having asthma during the last half hour? (Yellow Card)
### RESPONSES

<table>
<thead>
<tr>
<th>RESPONSE</th>
<th>1st</th>
<th>2nd</th>
<th>3rd</th>
</tr>
</thead>
<tbody>
<tr>
<td>shortness of breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>coughing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>afraid of not having your asthma medications available</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>fighting for air</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>frustrated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>concerned about the need to use medications for your asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chest tightness or chest heaviness</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>wheezing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>difficulty breathing out</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>afraid of getting out of breath</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>concerned about having asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### DOMAIN CODE:

- Symptoms: 1, 2, 4, 7, 8, 9
- Emotional Function: 3, 5, 6, 10, 11
RESPONSE OPTION CARDS

RED CARD
1. Extremely Bothered
2. Very Bothered
3. Quite Bothered
4. Somewhat Bothered
5. Bothered a bit
6. Hardly Bothered at all
7. Not bothered

YELLOW CARD
1. All of the time
2. Most of the time
3. Quite often
4. Some of the time
5. Once in a while
6. Hardly any of the time
7. None of the time
MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE (MiniAQLQ)

INTERVIEWER-ADMINISTERED

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For further information:

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Development and validation supported by
GLAXO WELLCOME, INC.

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OCTOBER 2000
Please complete all questions by circling the number that best describes how the patient has been during the last 2 weeks as a result of their asthma.

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

<table>
<thead>
<tr>
<th></th>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>Hardly Any of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Feel SHORT OF BREATH as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>2.</td>
<td>Feel bothered by or have to avoid DUST in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>3.</td>
<td>Feel FRUSTRATED as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4.</td>
<td>Feel bothered by COUGHING?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>5.</td>
<td>Feel AFRAID OF NOT HAVING YOUR ASTHMA MEDICATION AVAILABLE?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>6.</td>
<td>Experience a feeling of CHEST TIGHTNESS or CHEST HEAVINESS?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>7.</td>
<td>Feel bothered by or have to avoid CIGARETTE SMOKE in the environment?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>8.</td>
<td>Have DIFFICULTY GETTING A GOOD NIGHT’S SLEEP as a result of your asthma?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>9.</td>
<td>Feel CONCERNED ABOUT HAVING ASTHMA?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>10.</td>
<td>Experience a WHEEZE in your chest?</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>
MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE

INTERVIEWER-ADMINISTERED

DATE

IN GENERAL, HOW MUCH OF THE TIME DURING THE LAST 2 WEEKS DID YOU:

<table>
<thead>
<tr>
<th>All of the Time</th>
<th>Most of the Time</th>
<th>A Good Bit of the Time</th>
<th>Some of the Time</th>
<th>A Little of the Time</th>
<th>Hardly Any of the Time</th>
<th>None of the Time</th>
</tr>
</thead>
</table>

11. Feel bothered by or have to avoid going outside because of WEATHER OR AIR POLLUTION?

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

HOW LIMITED HAVE YOU BEEN DURING THE LAST 2 WEEKS DOING THESE ACTIVITIES AS A RESULT OF YOUR ASTHMA?

<table>
<thead>
<tr>
<th>Totally Limited</th>
<th>Extremely Limited</th>
<th>Very Limited</th>
<th>Moderate Limitation</th>
<th>Some Limitation</th>
<th>A Little Limitation</th>
<th>Not at all Limited</th>
</tr>
</thead>
</table>

12. STRENUIOUS ACTIVITIES (such as hurrying, exercising, running up stairs, sports)

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

13. MODERATE ACTIVITIES (such as walking, housework, gardening, shopping, climbing stairs)

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

14. SOCIAL ACTIVITIES (such as talking, playing with pets/children, visiting friends/relatives)

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

15. WORK RELATED ACTIVITIES* (tasks you have to do at work)

   | 1 | 2 | 3 | 4 | 5 | 6 | 7 |

*If the patient is not employed or self-employed, these should be tasks they have to do most days.

DOMAIN CODE:

Symptoms: 1, 4, 6, 8, 10
Activity Limitation: 12, 13, 14, 15
Emotional Function: 3, 5, 9
Environmental Stimuli: 2, 7, 11
### MINI ASTHMA QUALITY OF LIFE QUESTIONNAIRE

<table>
<thead>
<tr>
<th>BLUE CARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. ALL OF THE TIME</td>
</tr>
<tr>
<td>2. MOST OF THE TIME</td>
</tr>
<tr>
<td>3. A GOOD BIT OF THE TIME</td>
</tr>
<tr>
<td>4. SOME OF THE TIME</td>
</tr>
<tr>
<td>5. A LITTLE OF THE TIME</td>
</tr>
<tr>
<td>6. HARDLY ANY OF THE TIME</td>
</tr>
<tr>
<td>7. NONE OF THE TIME</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GREEN CARD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. TOTALLY LIMITED, COULDN’T DO ACTIVITY AT ALL</td>
</tr>
<tr>
<td>2. EXTREMELY LIMITED</td>
</tr>
<tr>
<td>3. VERY LIMITED</td>
</tr>
<tr>
<td>4. MODERATE LIMITATION</td>
</tr>
<tr>
<td>5. SOME LIMITATION</td>
</tr>
<tr>
<td>6. A LITTLE LIMITATION</td>
</tr>
<tr>
<td>7. NOT AT ALL LIMITED</td>
</tr>
</tbody>
</table>
References


AZALEA Post Analysis Report

Chief Investigator: Professor Sebastian L Johnston

EudraCT NUMBER: 2011-001093-26
SPONSOR: Imperial College Academic Health Science Centre
FUNDER: NIHR, Efficacy and Mechanism Evaluation Programme
STUDY COORDINATION CENTRE: Imperial Clinical Trials Unit
Date: 05/04/2016

Prepared by Matyas Szigeti (Trial Statistician)
1 PRE – SPECIFIED ANALYSES

All analysis specified in the Statistical Analysis Plan (SAP) for all primary and secondary outcomes were carried out, except subgroup analysis based on sputum cell count characteristics as numbers per group were too low to be meaningful.

2 SAP AMENDMENT ON DEFINITION OF DAY 1

Day 1 was defined in the Statistical Analysis Plan (SAP) as “the Day 1 diary card of each patient will be defined to be the first diary is completed within 24 hours of randomization”. This definition was not applicable, because the times of diary cards were not recorded in InForm (nor the time of randomisation), therefore if the date was the day after the randomization it is not possible to determine if that was within 24 hours or not.

After careful consideration, the study team agreed to amend the definition of Day 1 to the following: “the day of the administration of the first dose of study drug, or if it is not available, then the day of the randomization. If no diary card has been completed on that day, their Day 1 diary card will be treated as missing. Diary cards for Days 2-10 will be determined in a similar way.”

Date of the study drug admission was available for all randomised patients.

This has no effect on the secondary outcomes as both AQLQ, miniAQLQ and pulmonary function test were taken only three times and the analysis is based on the order of visits not on the days.