A Single-Center, Double-Blind, Randomized, Placebo-Controlled Crossover Study to Evaluate the Effect of Solithromycin on Airway Inflammation in Male and Female Patients with Chronic Obstructive Pulmonary Disease

Protocol Number:	CE01-204
Study No:	CE01-204
Study Phase:	2
Study Initiation Date:	29 September 2015 (first screening visit)
Study Completion Date:	27 March 2017 (JRCO closeout letter)
Medical Officer:	Craig Batista, MBBS, BSc, MRCP, FHEA
Principal Investigator:	Peter J. Barnes, MA, DM, DSc, FRCP, FCCP, FMedSci, FRS
Date of Report:	
Version:	Version 1

Cempra Pharmaceuticals, Inc.
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2 SYNOPSIS

Name of Sponsor Company Imperial College London	**Name of Finished Product**	**Name of Active Ingredients**

TITLE OF STUDY:	A Single-center, Double-blind, Randomized, Placebo-controlled Crossover Study to Evaluate the Effect of Solithromycin on Airway Inflammation in Male and Female Patients with Chronic Obstructive Pulmonary Disease
STUDY NO:	CE01-204
CLINICAL PHASE:	2
INVESTIGATOR(S):	Dr. Peter J Barnes, Dr. Craig Batista, Dr. Louise Donnelly
STUDY CENTER(S):	Single-center trial, Imperial College London – Royal Brompton Hospital
PUBLICATION:	A Single-center, Double-blind, Randomized, Placebo-controlled Crossover Study to Evaluate the Effect of Solithromycin on Airway Inflammation in Male and Female Patients with Chronic Obstructive Pulmonary Disease

STUDY PERIOD:

First Subject Enrolled: 13 November 2015 (first dose)

Last Subject Completed: 18 February 2016 (last visit)

OBJECTIVES:

The primary objective was:

- To assess the number of sputum neutrophils per mL in patients treated with solithromycin compared to patients treated with placebo

The secondary objectives were:

- To assess concentrations of sputum CXCL8, IL-6, MPO, MMP-9, MCP-1 and TNF-α and nasal CXCL8 in patients treated with solithromycin compared to patients treated with placebo
- To assess FEV1, FVC, R5, and the COPD Assessment Test (CAT) score in patients treated with solithromycin compared to patients treated with placebo
- To assess the safety and tolerability of oral solithromycin in adult patients with COPD

The exploratory objectives were:

- To assess the activity of HDAC2, PI3K, NF-κB in sputum macrophages in patients treated with solithromycin compared to patients treated with placebo and to assess the levels of serum biomarkers (fibrinogen, C-reactive protein) in patients treated with solithromycin compared to patients treated with placebo
- Additional cytokines/chemokines or biomarkers may be added as exploratory endpoints if new information develops.

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METHODOLOGY:

Study Design:

This was a Phase 2, single-center, double-blind, randomized, placebo-controlled crossover study to evaluate the effect of solithromycin on airway inflammation in male and female patients with COPD.

Patients who signed the informed consent and met all inclusion and exclusion criteria were administered 28 days of solithromycin 400 mg PO QD or matching placebo, followed by a 28-day washout period, then crossed over to receive either placebo or solithromycin for another 28 days. Sputum, nasal and blood samples were collected and assayed for various inflammatory markers at baseline and after each 4-week treatment.

Study Duration:

The study duration from first patient first visit until last patient last visit was approximately 4 months

Subject Participation:

The duration of individual patient participation from enrollment until study completion was approximately 105 days, including Screening up to 21 days prior to dosing, 28 days of dosing in Treatment Period 1, a 28-day washout period, and 28 days of dosing in Treatment Period 2.

NUMBER OF SUBJECTS:

6 subjects were enrolled but only 5 were randomized into CE01-204 before it was terminated. A single subject was withdrawn between enrollment and randomization, due to an exacerbation of COPD.

DIAGNOSIS AND MAIN CRITERIA FOR INCLUSION:

Inclusion Criteria

1. Male and female patients ≥ 45 years of age.
2. History of cigarette smoking >10 pack-years.
3. Post-bronchodilator FEV1/FVC of <0.70 and FEV1 of 30-79% of predicted normal value.
4. Patients on prescribed inhaled corticosteroids can be enrolled.
5. Females of non-childbearing potential: surgically sterile (e.g. tubal ligation) or at least 2 years post-menopausal.
6. Females of childbearing potential (including females less than 2 years post-menopausal) must have a negative pregnancy test at enrollment and must agree to use highly effective methods of birth control (i.e. diaphragm plus spermicide or male condom plus spermicide, oral contraceptive in combination with a second method, contraceptive implant, injectable contraceptive, indwelling intrauterine device, sexual abstinence, or a vasectomized partner) while participating in the study and for 30 days after the last dose of study drug. Only true sexual abstinence can be accepted as a contraceptive method (in line with preferred and usual lifestyle of the patient). Period abstinence (e.g. calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea (LAM) are not acceptable methods of contraception.
7. Males who wish to father children can be enrolled.
8. The patient must be willing and able to comply with all study visits and procedures.
9. The patient must be a suitable candidate for oral therapy and be able to swallow capsules intact.
10. The patient must provide written informed consent.
11. No evidence of active bacterial infection in sputum by qPCR evaluation.

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Exclusion Criteria

1. Acute exacerbation of COPD within the previous 60 days or during the washout period of the study.
2. Any condition that could possibly affect oral drug absorption, e.g. gastroenteritis, status post gastrectomy, status post bariatric surgery.
3. Currently taking medication for HIV, chronic hepatitis B, or hepatitis C virus (HCV) infection.
4. Currently taking theophylline or other xanthine medication.
5. Currently taking warfarin.
6. Known concomitant infection (pulmonary or otherwise) which would require additional systemic antibiotics.
7. QTc greater than 450 msec in males or females as corrected by the Fridericia formula.
8. Current use of drugs known to prolong the QT interval, including Class Ia (quinidine, procainamide) or Class III (amiodarone, sotalol) antiarrhythmics.
9. Concomitant use of drugs, foods, or herbal products known to be moderate to potent inhibitors of CYP3A4 isozymes: oral antifungal agents (e.g. ketoconazole, itraconazole, posaconazole, fluconazole and voriconazole); HIV protease inhibitors (e.g. ritonavir and saquinavir), HCV protease inhibitors (e.g. boceprevir and telaprevir), nefazodone, fluvoxamine, conivaptan, diltiazem, verapamil, aprepitant, ticlopidine, crizotinib, imatinib; grapefruit or grapefruit juice.
10. Any use within the prior 7 days of drugs or herbal products known to be moderate to potent inducers of CYP3A4 isozymes: St. John's Wort, rifampin, rifabutin, anti-convulsants (e.g. phenobarbital, carbamazepine, phenytoin, rufinamide), modafinil, armodafinil, etraverine, efavirenz, bosentan.
11. Required current use of drugs with narrow therapeutic indices that are principally metabolized by CYP3A4 or transported by P-glycoprotein (P-gp), for which a drug interaction with solithromycin could result in higher and possibly unsafe exposures to these drugs: e.g. the P-gp substrates digoxin or colchicine and the CYP3A4 substrates alfentanil, astemizole, cisapride, cyclosporine, dihydroergotamine, ergotamine, fentanyl, midazolam, pimozide, quinidine, sirolimus, tacrolimus, everolimus, and terfenadine).
12. History of organ transplant.
12. Cytotoxic chemotherapy or radiation therapy within the previous 3 months.
13. Known neuromuscular disorder from clinical history (e.g. myasthenia gravis, Parkinson's disease).
14. Known significant renal, hepatic, or hematologic impairment.
15. Women who are pregnant or breast feeding.
16. Prior participation in this protocol.
17. Any investigational drugs taken or investigational devices used within 4 weeks before administration of the first dose of the study drug.
18. History of intolerance or hypersensitivity to macrolide antibiotics.
19. Any concomitant condition that, in the opinion of the Investigator, would preclude an evaluation of a response or make it unlikely that the contemplated course of therapy and follow-up could be completed (e.g. life expectancy <30 days).

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TEST PRODUCT

Dose and Mode of Administration: Solithromycin (CEM-101) 400 mg QD (2×200 mg capsules)

Duration of Treatment: 28 days crossover

Batch Number(s): Bulk: 1402288, Packaged lot: 9009644-005

REFERENCE THERAPY:

Dose and Mode of Administration: Placebo to match 2 capsules PO QD

Duration of Treatment: 28 days crossover

Batch Number(s): Bulk: 1402286, Packaged lot: 9019117-001B

CRITERIA FOR EVALUATION

Efficacy:

The primary efficacy outcome measure was the number of sputum neutrophils per mL with solithromycin treatment compared to placebo. A differential cell count was performed on fixed cytospin preparations stained with Diff-Quick. Comparisons were made between the pre- and post- treatment values, including the number of neutrophils per ml.

Secondary efficacy outcomes included the following:

- Concentrations of sputum CXCL8, IL-6, MPO, MMP-9, MCP-1 and TNF-α and nasal CXCL8 with solithromycin treatment compared to placebo
- FEV1, FVC, R5, and the COPD Assessment Test (CAT) score with solithromycin treatment compared to placebo

Planned exploratory efficacy outcomes included the following:

- Sputum macrophages (>0.5 x 10^6) isolated by adhesion and assayed for HDAC2, PI3K (Akt phosphorylation) and NF-κB activity.
- Serum samples assayed for fibrinogen and C-reactive protein.
- Nasal epithelial lining fluid assayed for cytokines. Nasal epithelial cells analyzed for NF-κB activation.
- Additional cytokines/chemokines or biomarkers could be added as exploratory endpoints if new information developed.

Pharmacokinetics:

Plasma samples for determination of drug concentrations were obtained from all subjects approximately 4 hours post dose on Days 7, 14, and 28 (±2 Days) of each Period. Samples from solithromycin recipients were analyzed by the unblinded bioanalytical laboratory. The results were used to confirm therapeutic drug exposure and were not sufficient to determine pharmacokinetic parameters for solithromycin and metabolites.

Safety:

Safety was evaluated in the Safety population by presenting summaries of AEs, routine clinical laboratory evaluations, and vital signs. Subjects who received at least one dose of study drug wereincluded in the safety population.

Summary tables were provided for all TEAEs. A TEAE was defined as an AE with a start date and time on or after start of administration of study drug. Adverse events were coded using the Medical Dictionary for Regulatory Activities (MedDRA®). The number and percentage of subjects with TEAEs were tabulated by system organ class and MedDRA preferred term. Incidence was summarized separately for all TEAEs and for study drug related AEs. Severity of AEs were summarized similarly. Adverse events leading to premature discontinuation from the study drug and serious TEAEs were presented in Table 19.

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Mean changes from baseline at the Day 7, 14, and 28 (±2 days) evaluations for clinical laboratory parameters were summarized using descriptive statistics. The number and percentage of subjects with treatment-emergent potentially clinically significant (PCS) laboratory values were tabulated. Treatment-emergent PCS laboratory tests were those in which the baseline value was not PCS and the post-baseline value was PCS. PCS were defined based on the Division of Microbiology and Infectious Diseases (DMID) criteria.

Mean changes from baseline at the Day 7, 14, and 28 (±2 Days) evaluations of blood pressure and heart rate were summarized. The number and percent of subjects with treatment-emergent PCS values were tabulated.

Statistical Methods:

Data were to be described and analyzed using the Prism software, Version 6.0 or higher (GraphPad Inc., USA). Individual subject data are presented in subject case report forms. Descriptive statistics including number of subjects (N), mean, standard deviation (SD), standard error of the mean (SEM), median, and minimum and maximum were presented for continuous data. For categorical data, frequency and percentage of subjects in each category were presented.

RESULTS

11 subjects were screened, six were enrolled, five received study drug and one subject (003) was withdrawn for an acute COPD exacerbation before receiving study drug.

Efficacy Results:

The study was terminated early and therefore formal data analysis could not be performed due to the low number of recruited subjects. As such, only selected outcome measures (primary, secondary and exploratory) were assessed. These included: sputum neutrophils per ml (primary outcome), sputum CXCL8, nasal CXCL8, FEV1, R_5, CAT, CRP and fibrinogen.

A trend towards a reduction in sputum neutrophils was observed with solithromycin, but not placebo. There appears to have been a trend toward reduction in sputum and nasal epithelial lining fluid CXCL8 concentrations. In each case, due to the small sample size, no statistical comparisons have been made, and no firm conclusions can be drawn.

There was no evidence of an effect of solithromycin on FEV1 (in L or % of predicted value), airway resistance, COPD assessment test results, or plasma CRP and fibrinogen levels.

Pharmacokinetic Results:

Plasma concentrations of solithromycin (CEM-101) were measured from all subjects approximately 4 hours post dose on Days 7, 14, and 28 (±2 Days) of each Treatment Period. Plasma concentrations of solithromycin (CEM-101) and the two major metabolites, N-Acetyl-CEM-102 and CEM-214 demonstrated considerable interindividual and intraindividual variability. Observed CEM-101 C_{max} values ranged from 321 to 1,200 ng/mL. There was no evidence of plasma accumulation of CEM-101 over the 4 weeks of treatment with daily doses of 400 mg. Plasma CEM-101 levels in the subject with cholestatic hepatitis were not elevated at the time of that event.

No formal PK analyses were performed, given the limited number of subjects enrolled.

Safety Results:

Four subjects received solithromycin during this trial. In three, ALT elevation to >3xULN was observed during or shortly after solithromycin dosing. In one subject, an episode of cholestatic hepatitis occurred, with associated icterus and pruritus. There was no loss of hepatic function (as measured by PT/INR and clinical parameters), and the subject recovered rapidly off study drug. This event led to cessation of recruitment and later to discontinuation of the study.

Other observed adverse events included transient gastrointestinal events (nausea, reflux, abdominal pain) considered to be of mild severity.

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CONCLUSIONS: The trial was terminated early due to the observation of cholestatic hepatitis in a single subject, and marked ALT elevation in two others, during or following solithromycin dosing. Data from the four subjects taking solithromycin suggested a trend towards reduced sputum neutrophil numbers and CXCL8 levels, however, the data set is too small for analyses.		
DATE OF THE REPORT: 6 November 2017		

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4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE	adverse event
CFR	Code of Federal Regulations
C_{max}	maximum concentration in plasma
CAT	COPD Assessment Test
CRF	case report form
ECG	electrocardiogram
FDA	U.S. Food and Drug Administration
FEV1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GOLD	Global Initiative for Chronic Obstructive Lung Disease (GOLD). Refers to criteria for assessing mortality and COPD exacerbation risk.
Hz	Hertz, a measure of frequency (1 Hz = 1 cycle/second)
IOS	Impulse Oscillometry System (a measure of airway resistance)
IRB	Institutional Review Board
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
NCI-CTCAE	National Cancer Institute Common Toxicity Criteria for Adverse Events
PK	pharmacokinetics
SAE	serious adverse event
SAP	Statistical Analysis Plan
SOC	system organ class
$t_{1/2}$	half-life
t_{max}	time to maximum concentration in plasma
WHO	World Health Organization

5 ETHICS

The protocol, subject information sheets, informed consent forms and all relevant supporting documents and data were submitted to a National Research Ethics Committee (Bloomsbury, London; Reference 13/LO/1403) and the Medicines and Healthcare Products Regulatory Agency (MHRA) for approval. Approval was obtained before recruitment and study initiation. Appropriate clinical trial insurance was held. Study monitoring was undertaken by the Joint Research Compliance Office (JRCO) at Imperial College. The following references applied:

Research ethics committee (REC):14/LO/2066

Protocol number: CE01-204

EudraCT number: 2014-003077-42

6 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

Table 1 Study Administrative Structure

Person/Organization	Address	Service Provided
Peter J. Barnes, MA, DM, DSc	Imperial College Dovehouse Street London, SW3 6LY	Sponsor Principal Investigator
Louise Donnelly, BSc PhD	Imperial College Dovehouse Street London, SW3 6LY	Co-Investigator
Craig Barnes, MBBS, BSc	Imperial College Dovehouse Street London, SW3 6LY	Co-Investigator
David Oldach, MD Chief Medical Officer	Cempra Pharmaceuticals, Inc. 6340 Quadrangle Drive, Suite 100 Chapel Hill, NC 27517	Cempra Medical Officer (Conduct, Analysis, Report)
Amanda Bravery, InForm, Imperial College	Head of ICT – ICTU, 1st, Stadium House, White City	eCRF and Data Management
Jocelyn Mora	Imperial College London	Monitoring
MicroConstants, Inc.	9050 Camino Santa Fe San Diego, CA 92121	Bioanalytical Analysis of Plasma and Serum Samples
Catalent Pharma Solutions, Inc.	1100 Enterprise Drive Winchester, KY 40391 USA Clinical Supplies Services 3031 Red Lion Road Philadelphia, PA 19114 USA	Manufacturing and Distribution of Clinical Supplies

7 INTRODUCTION

Inflammation in COPD. COPD is associated with chronic inflammation affecting peripheral airways and lung parenchyma that leads to progressive and largely irreversible airway obstruction. This inflammation is progressive even when smoking is stopped and is unresponsive even to high doses of corticosteroids. At present there are no safe and effective anti-inflammatory therapies which have resulted in significant reductions in disease progression or mortality [1,2]. There is a pressing need to discover novel treatments that target COPD inflammation or that reverse corticosteroid resistance by targeting the molecular pathways of resistance. An effective anti-inflammatory therapy should reduce disease progression, exacerbations and comorbidities that are linked to systemic inflammation and mortality. The inflammation in COPD is largely driven through the activation of the pro-inflammatory transcription factor nuclear factor-κB (NF-κB), particularly in macrophages and respiratory epithelial cells [3,4]. NF-κB activation results in increased histone acetylation which is in turn associated with activation of multiple inflammatory genes, including TNF-α, IL-6, CXCL8 and MMP-9. The increased oxidative stress in COPD also activates phosphoinositide-3-kianse δ (PI3Kδ), which in turn inactivates the nuclear enzyme histone deacetylase (HDAC)-2 via phosphorylation [5,6]. Theophylline, which in low concentrations inhibits oxidant-activated PI3Kδ, is able to increase HDAC2 in COPD cells and thus, to an extent, reversing corticosteroid resistance [5].

Macrolides in COPD. Macrolides have been used to treat acute exacerbations of COPD, 50% of which are caused by bacterial infection (mainly *Haemophilus influenzae* and *Streptococcus pneumoniae*), but long-term macrolide therapy has proved to be useful in treating bronchiectasis and cystic fibrosis, which involve chronic neutrophilic inflammation of the airways. Recently long-term macrolide therapy with erythromycin and azithromycin has been shown to reduce exacerbations in COPD patients [7,8]. However, it is not clear whether this effect is due to the anti-bacterial or the anti-inflammatory effects of macrolides. Measurement of inflammatory mediators in sputum did not show any reduction with erythromycin, although this macrolide has a relatively weak anti-inflammatory profile. It is possible that the improvement is due to reduced bacterial colonisation of the lower airways (mainly *H influenzae*, *S pneumoniae* and *Moraxhella cattarhalis*), which is found in 30-50% of COPD patients.

Solithromycin. Solithromycin (CEM-101) is a novel macrolide and the first fluoroketolide. It is active against Gram-positive, Gram-negative and atypical bacteria and is in clinical development for the treatment of community-acquired bacterial pneumonia and gonorrhoea. It inhibits bacterial synthesis by binding to multiple sites on the 23S component of the 50S ribosome (creating a greater hurdle for development of antibiotic resistance in target pathogens), whereas other macrolides bind at a single site. Development of solithromycin resistance has not been observed to date in clinical trials and solithromycin is active against bacterial strains that are resistant to other macrolides. It is 8-16 times more active than azithromycin and has a one day duration of action. It does not activate the motilin receptors in the gastrointestinal tract which has been linked to the induction of diarrhoea by other macrolides. In clinical studies oral solithromycin appeared to be generally well tolerated in the treatment of CABP with diarrhoea and elevated liver enzymes as the most frequently observed adverse events [9,10]. Solithromycin has an effect on the hERG channel but in clinical study has had no significant effects on QTc or cardiac rhythm.

In a small clinical Phase 1 study, oral solithromycin (400 mg) once daily was substantially concentrated in lung epithelial lining fluid (~10-fold) and alveolar macrophages (~200-fold) compared to plasma concentrations, indicating its suitability for local effects in the lung [11]. Solithromycin was found to have greater anti-inflammatory effects than other macrolides, such as erythromycin, azithromycin, clarythromycin and telithromycin (10-fold greater effect) in suppressing TNF-α, CXCL8 and MMP-9 release and activity from a human macrophage cell line and from monocytes from COPD patients [12]. In addition solithromycin completely inhibited oxidative stress-activated NF-κB in these cells. Furthermore, solithromycin was effective in suppressing cigarette smoke-induced neutrophilic inflammation and MMP-9 release in mice *in vivo*, whereas erythromycin was ineffective. In addition, solithromycin increased HDAC2 after exposure to oxidative stress and reversed corticosteroid resistance through an effect that involved inhibiting the PI3K signalling pathway [13], indicating that, as well as a direct anti-inflammatory effect through NF-κB inhibition, it may also reverse corticosteroid resistance in COPD.

These observations suggest that oral solithromycin may have anti-inflammatory effects in COPD both by a direct anti-inflammatory effect on macrophages, but also by reversing corticosteroid resistance and thus restoring the anti-inflammatory effects of inhaled corticosteroids.

8 STUDY OBJECTIVES

Primary:

- To assess the number of sputum neutrophils per mL in patients treated with solithromycin compared to patients treated with placebo

Secondary:

- To assess concentrations of sputum CXCL8, IL-6, MPO, MMP-9, MCP-1, TNF-α and nasal CXCL8 in patients treated with solithromycin compared to patients treated with placebo
- To assess forced expiratory volume at 1 second (FEV1), forced vital capacity (FVC), resistance at 5Hz (R5), and the COPD Assessment Test (CAT) score in patients treated with solithromycin compared to patients treated with placebo
- To assess the safety and tolerability of oral solithromycin in adult patients with COPD

Exploratory Objective:

- To assess the activity of HDAC2, PI3K, NF-κB in sputum macrophages in patients treated with solithromycin compared to patients treated with placebo and to assess the levels of serum biomarkers (fibrinogen, C-reactive protein) in patients treated with solithromycin compared to patients treated with placebo.
- Additional cytokines/chemokines or biomarkers may be added as exploratory endpoints if new information develops.

9 STUDY DESIGN

9.1 Overall Study Design and Schedule of Assessments

This was a Phase 2, single-center, double-blind, randomized, placebo-controlled crossover study to evaluate the effect of solithromycin on airway inflammation in male and female patients with COPD.

Patients who sign the informed consent and meet all inclusion and exclusion criteria were administered 28 days of solithromycin 400 mg PO QD or matching placebo, followed by a 28-day washout period, then crossover to receive either placebo or solithromycin for another 28 days. Sputum, nasal and blood samples were collected and assayed for various inflammatory markers at baseline and after each 4-week treatment (Table 2).

9.1.1 Duration of Subject Participation

The duration of individual patient participation from enrollment until study completion was approximately 105 days, including Screening up to 21 days prior to dosing, 28 days of dosing in Treatment Period 1, a 28-day washout period, and 28 days of dosing in Treatment Period 2.

9.1.2 Number of Subjects

Enrollment of up to 36 subjects was planned to ensure that at least 30 subjects completed the two treatment periods and could be evaluated for treatment effect. Five subjects were enrolled into the trial before it was terminated.

9.1.3 Study Procedures

The schedule of assessments follows, See Table 2. For additional details, please refer to the study protocol.

Table 2 Schedule of Assessments and Procedures

Activities		Treatment Periods 1 and 2						
	Day -21 Screening	Baseline [a]	Day 1	Day 7 [±2 Days]	Day 14 [±2 Days]	Day 28 [±2 Days]	28-Day Washout [b]	End of Study Follow-up Call [m]
Informed Consent	X							
Medical History/ Prior Medications	X							
Directed Physical Examination, Height and Weight [c]	X					X		
Vital signs [d]	X	X		X	X	X		
ECG [e]	X							
Induced Sputum [f]	X	X				X		
Pulmonary function tests and CAT score [g]		X				X		
Nasal sampling [h]		X				X		
Clinical Laboratories – FBC, Biochemistry [i]	X	X		X	X	X		
Serum pregnancy test [j]	X	X				X		
PK Samples [k]				X	X	X		
Serum sample for biomarkers [l]		X			X	X		
Collect and record AEs		X	X	X	X	X	X	X
Concomitant Medications/ Treatments/Procedure		X	X	X	X	X	X	
Study Drug Administration			X					
Perform Study Drug Accountability			X	X	X	X		

a. Baseline for Treatment Period 1 could be the same day as Screening. Baseline for Period 2 will occur during the washout period within 7 days prior to study drug administration in Treatment Period 2.
b. A total of 28 days between the last dose of study drug in Treatment Period 1 (Day 28) and the first dose of study drug in Treatment Period 2 (Day 1). Patients were contacted two weeks into the washout period (±2 days) to assess items listed above during this time. Patients should be instructed to bring the completed diary with them upon return to the site to begin Treatment Period 2.
c. Directed physical examination to include: lungs, heart, and abdomen.
d. Vital signs to include: heart rate, blood pressure, temperature, respiratory rate and pulse oximetry.
e. At Screening all participants received a 12-lead standard electrocardiogram.
f. Induced sputum using nebulized saline. At screening only, a portion of the sputum sample underwent qPCR testing to identify active bacterial infections.
g. To include: FEV_1, FVC, R_5 and CAT score
h. A synthetic absorption matrix to measure nasal epithelial lining fluid for cytokine assays and nasal microcurette for epithelial cell sampling for NF-κB activation.
i. The following laboratory tests were done at Baseline and Days 7, 14, and 28 in both Treatment Period 1 and Treatment Period 2.: Serum Chemistry (creatinine, blood urea nitrogen, alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), total and direct bilirubin, albumin, total protein, glucose, sodium, potassium, chloride, calcium, and phosphorus, and creatine phosphokinase); Hematology (full blood count with differential).

j. All females of childbearing potential had a serum pregnancy test at Baseline and Day 28 in both Treatment Period 1 and Treatment Period 2
k. Blood was collected for determination of solithromycin concentrations at approximately 4 hours post-dose on Days 7, 14, and 28.
l. Serum sample to evaluate levels of fibrinogen and C-reactive protein.
m. Patients were contacted at 2 weeks [±2 days] following their last day of study participation post Treatment Period 2 to assess patient's status.

9.2 Changes in the Conduct of the Study or Planned Analyses

9.2.1 Amendment #1 – 29 January 2015

Amendment 1 was introduced after MHRA review, to update the reference safety information, to include additional criteria for drug discontinuation and to ensure appropriate reporting of SAEs. Appendix B was added to include safety information from the Phase 2 CAPB study with a listing of serious adverse drug reactions. The study procedures included new drug discontinuation criteria based on laboratory findings of increased hepatic transaminases, QT prolongation > 500 ms or change from baseline > 60 ms, increases in heart rate > 120 bpm or change from baseline > 30 bpm, and adverse events associated with inhibition of nicotinic acetylcholine receptors. Additional detail was incorporated to describe procedures for reporting of SAEs to regulatory authorities.

10 STUDY SUBJECTS

10.1 Disposition of Subjects

Eleven patients were screened at a single center in the United Kingdom (Imperial College London - Royal Brompton & Harefield Foundation Trust). Six subjects were enrolled, five received study drug (either solithromycin or placebo or both) and one subject (003) was withdrawn for an acute COPD exacerbation before receiving study drug. The first subject was enrolled on 13 November 2015 and the final study visit for any enrolled patient was 18 February 2016. Four subjects received solithromycin and four subjects received placebo. One subject discontinued study drug dosing during Treatment Period 1 (001; solithromycin) due to an elevation of hepatic transaminases. One subject discontinued the study after Treatment Period 1 (002; placebo) due to a COPD exacerbation. Three subjects completed efficacy evaluations while randomized to the solithromycin and four subjects completed the evaluations while randomized to placebo presented in Table 3.

Table 3 Disposition of Subjects

Subject Disposition	Overall n (%)	Placebo n (%)	Solithromycin 400 mg n (%)
Subjects Randomized	6	6	6
Safety Population	5 (83.3)	4 (66.7)	4 (66.7)
PK Population	5 (83.3)	4 (66.7)	4 (66.7)
Subjects Completed	3 (50.0)	4 (66.7)	3 (50.0)
Subjects Discontinued	3 (50.0)	2 (33.3)	2 (33.3)
Discontinuation Reason:			
Adverse Event	3 (50.0)	1 (16.7)	1 (16.7)

10.2 Protocol Deviations

A full list of all protocol deviations is presented in Table 4. None of the protocol deviations affected evaluability.

Table 4 Major Protocol Deviations

Date of Deviation	Deviation Type [a]	Deviation Description	Major/Minor
30/10/15	3	Biochemistry sample not run for glucose by the hospital laboratory despite being requested. Sample discarded by lab, so could not be run at a later date (Subject 4)	Minor
30/10/15	3	Biochemistry sample not run for glucose by the hospital laboratory despite being requested. Sample discarded by lab, so could not be run at a later date (Subject 5)	Minor
11/12/15	3	Assay buffer not available on site for nasal sample. Added once the sample was received at the NHLI (Subject 2)	Minor
11/12/15	3	Subject visit not within the specific ± 2 days due to weekends and subject schedule (Subject 2)	Minor

Date of Deviation	Deviation Type [a]	Deviation Description	Major/Minor
25/01/16	3	Subject visit not within the specific ± 2 days due to weekends and subject schedule (Subject 6)	Minor
05/02/16	3	Subject visit not within the specific ± 2 days due to weekends and subject schedule (Subject 5)	Minor

a. 1 = Inclusion/Exclusion criteria; 2 = Prohibited Con Meds; 3 = Subject not managed per protocol (e.g., out of window visits, missed procedures); 4 = Incorrect dosing/dosing issue; 5 = Other

10.3 Demographic and Other Baseline Characteristics

10.3.1 Demographics

Demographic and baseline characteristics are presented in Table 5. All subjects (3 male / 3 female) were Caucasian (6; 100%) and were of non-Hispanic ethnicity. Age ranged from 64 to 75, weight ranged from 50.2 to 108 kg, and BMI ranged from 19.6 to 40.1 kg/m^2.

10.3.2 Baseline Characteristics

Table 5 Demographic Characteristics.

Parameter	Baseline Mean (SD) (N=6)	Range (N=6)
Age (y)	69.3 (3.6)	64 - 75
Gender		
Male	3 (30)	na
Female	3 (30)	
Race		
Caucasian	6 (100)	na
Ethnicity		
Not Hispanic or Latino	6 (100)	na
Weight (kg)	75.2 (7.7)	50.2-108
Height (cm)	168.2 (3.4)	160 - 178
BMI (kg/m^2)	26.7 (3.0)	19.6 – 40.1

10.3.2.1 Medical History

All subjects had a prior diagnosis of COPD. Additional medical diagnoses among the 6 enrolled subjects are presented in Table 6.

Table 6 Past Medical History and Surgical History

Subject	Medial Diagnoses and Surgical Procedures (year of diagnosis) other than COPD
001	Cataracts (2002), retinal detachment (2003) and benign prostatic hypertrophy (2011)
002	Temporal arteritis (2003), appendectomy (2010), osteoarthritis (2010) and osteoporosis (2012)
003	Hysterectomy (1984), hiatus hernia (1996), bladder surgery (1997), cholecystectomy (2013) and rheumatoid arthritis (2015)
004	Arthritis (2000) hypercholesterolaemia (2001), abdominal hernia (2010)
005	Hypercholesterolaemia (2000), Hypothyroidism (2010), Ischaemic heart disease (2015).
006	None

10.3.2.2 Prior and Concomitant Medications

Relevant treatment [prior/current medications, including all prescription/non-prescription medications, herbal medications, and vitamin supplements, and prior/current non-pharmacological (surgery, procedures) treatments] received by the subject within 7 days before administration of study drug were recorded. All enrolled subjects were receiving long acting combination corticosteroid and β2-agonist inhaled preparations and 5 subjects received tiotropium bromide, a long-acting inhaled anticholinergic bronchodilator, for treatment of COPD. In addition to these treatments, 5 subjects received salbutamol, a short-acting β2-agonist inhaler, for rescue therapy. Other concomitant medications used by at least 2 subjects included omeprazole (n=3) and statins (n=2); See Table 7 and Table 8.

Table 7 Concomitant Medications (Oral)

Subject	Regular Oral Medication (Concomittant)		
001	Finasteride (5 mg PO OD)	-	-
002	Alendronic acid (70 mg PO OW)	Omeprazole (20 mg PO OD)	Calcium carbonate / vitamin D_3 (1.5 g/400 I.U. PO BD)
003	Omeprazole (10 mg PO OD)	Hydroxychloroquine (200 mg PO BD)	Naproxen (500 mg PO BD)
004	Omeprazole (20 mg PO OD)	Simvastatin (40 mg PO OD)	Naproxen (250 mg PO OD)
005	Levothyroxine (75 mcg PO OD)	Atorvastatin (20 mg PO OD)	-
006	-	-	-

Table 8 Concomitant Medications (Inhaled)

Subject	Regular Inhaled Medication (Concomittant)	
001	Fluticasone propionate / salmeterol (500 mcg / 50 mcg BD)	Tiotropium (5 mcg OD)
002	Budesonide/Formoterol (800 mcg / 24 mcg BD)	Tiotropium (18 mcg OD)
003	Fluticasone propionate / salmeterol (500 mcg / 50 mcg BD)	Tiotropium (18 mcg OD)
004	Fluticasone propionate / salmeterol (500 mcg / 50 mcg BD)	Tiotropium (18 mcg OD)
005	Fluticasone propionate / salmeterol (500 mcg / 50 mcg BD)	Tiotropium (18 mcg OD)
006	Fluticasone propionate / salmeterol (500 mcg / 50 mcg BD)	Tiotropium (18 mcg OD)

10.3.2.3 Disease Status at Baseline

Of the six subjects that undertook the study, the mean smoking history (± SEM) was 31.5 (± 4.1) pack years and all subjects were ex-smokers. The mean (± SEM) FEV_1, FEV_1 % predicted, FVC and FEV1 / FVC ratio were 1.29 L (± 0.14), 55.2 % (± 7.1), 2.87 L (± 0.33) and 0.48 (± 0.06), respectively. These results suggested moderate to severe airflow obstruction on average (GOLD stage 2 and 3). The mean R5 - R20 (± SEM) was 0.18 kPa.L-1.s (± 0.05). The mean CAT score (± SEM) was 17 (± 3) and the subjects experienced an average of 2 (± 0.6) exacerbations per year. All the subjects (except subject 002; Group C) were classified as Group D according to the combined GOLD assessment (i.e. more symptoms and high risk of exacerbation).

11 EFFICACY EVALUATION

The study was terminated early and therefore formal data analysis could not be performed due to the low number of recruited subjects. As such, only selected outcome measures (primary, secondary and exploratory) were assessed. These included: sputum neutrophils per ml (primary outcome), sputum CXCL8, nasal CXCL8, FEV1, R_5, CAT, CRP and fibrinogen. The following secondary outcome measures were not assessed: sputum concentrations of IL-6, MPO, MMP-9, MCP-1 and TNF-α. The following exploratory outcome measures were not assessed: the activity of HDAC2, PI3K and NFκB in sputum neutrophils.

11.1 Data Sets Analyzed

As detailed above, not all of the outcome measures were evaluated. All available data sets were analysed.

11.2 Measurements of Treatment Compliance

Pill counts were performed at each study visit. Treatment compliance was also confirmed ad hoc using PK results. All subjects were deemed to have been compliant throughout both 28 day treatment periods.

11.3 Efficacy Results

The primary outcome measure was the relative effects of solithromycin and placebo on the number of neutrophils per ml of sputum. These data for the subjects who completed 28 days of treatment with solithromycin or placebo are presented in Table 9. A trend towards a reduction in sputum neutrophils was observed with solithromycin, but not placebo. All subjects responded similarly. Due to the small sample size, no statistical comparisons have been made.

Table 9 Effect of placebo and solithromycin on sputum neutrophil counts

	Solithromycin Treatment Period Neutrophils x106/mL of Sputum			Placebo Treatment Period Neutrophils x106/mL of Sputum		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	3.83	4.57	0.74
004	4.06	1.35	-2.71	1.28	2.23	0.95
005	4.10	3.92	-0.18	3.38	7.58	4.20
006	3.43	2.14	-1.30	6.17	6.83	0.66
Mean	3.87 (± 0.22)	2.47 (± 0.76)	-1.39 (± 0.73)	3.66 (± 1.00)	5.30 (± 1.21)	1.64 (± 0.86)

Note: Subject 001 did not complete the first dosing period (solithromycin) due to an SAE (cholestatic hepatitis), with last dose of study drug received on Day 23. Subject 002 withdrew during the washout phase after receiving placebo in dosing period 1 (COPD exacerbation). Subject 003 was enrolled but never dosed with either placebo or solithromycin. Subjects 004, 005 and 006 received placebo and solithromycin. Induced sputum was collected at baseline (B) and day 28 (D28). Plugs were homogenised before being cytospin and fixation and staining of cells. Differential cell counts were then performed and differences between Baseline and Day-28 were calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

The effects of solithromycin and placebo on concentrations of CXCL8 in induced sputum supernatants were also assessed, as presented in Table 10, for the subjects who completed 28 days of either treatment. No trends were identified and due to the small sample size, no statistical comparisons were made.

Table 10 Effect of Placebo and Solithromycin on Sputum Concentrations of CXCL8

	Solithromycin Treatment Period Sputum CXCL8 Concentration (ng/mL)			Placebo Treatment Period Sputum CXCL8 Concentration (ng/mL)		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	4.92	4.88	-0.04
004	3.10	1.50	-1.60	3.64	3.63	-0.01
005	3.72	3.97	0.25	4.21	5.18	0.97
006	3.56	3.50	-0.06	3.64	3.47	-0.16
Mean	**3.46 (± 0.19)**	**2.99 (± 0.76)**	**- 0.47 (± 0.57)**	**4.10 (± 0.31)**	**4.29 (± 0.43)**	**0.19 (± 0.26)**

Note: (See Table 9 footnote regarding dosing history). Plugs were homogenised before centrifugation to obtain supernatants. Concentrations of CXCL8 were then measured using ELISA and differences between Baseline and Day-28 were calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

The effects of solithromycin and placebo on concentrations of CXCL8 in the nasal epithelial lining fluid were also evaluated, as shown in Table 11. Although there wasn't a clear trend for placebo, treatment with solithromycin appeared to reduce nasal CXCL8. No statistical comparisons were made.

Table 11 Effect of Placebo and Solithromycin on Nasal Epithelial Lining Fluid (NELF) CXCL8 Concentrations

	Solithromycin Treatment Period NELF CXCL8 Concentration (ng/mL)			Placebo Treatment Period NELF CXCL8 Concentration (ng/mL)		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	6.78	9.90	3.12
004	3.78	2.53	-1.24	1.90	3.42	1.52
005	3.48	0.60	-2.88	5.91	3.16	-2.75
006	1.18	1.04	-0.14	1.98	4.07	2.09
Mean	**2.81 (± 0.82)**	**1.39 (± 0.58)**	**-1.42 (± 0.80)**	**4.14 (±1.28)**	**5.14 (± 1.60)**	**1.00 (± 1.29)**

Note: (See Table 9 footnote regarding dosing history). Nasosorption was performed at baseline (B) and day 28 (D28). Concentrations of CXCL8 within the eluates were measured by ELISA and differences between B and D28 were calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

The effects of solithromycin and placebo on FEV_1 were evaluated, as presented in Table 12. There were no clear trends suggesting that either solithromycin or placebo affected FEV_1 (L) or FEV_1 (% predicted) during this dosing period. No statistical comparisons were made.

Table 12 Effect of placebo and solithromycin on FEV1

	Solithromycin Treatment Period FEV_1 liters (% predicted)			Placebo Treatment Period FEV_1 liters (% predicted)		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002: L (%)	-	-	-	0.94 (50%)	0.83 (44%)	-0.11 (-6%)
004: L (%)	0.97 (35%)	0.93 (34%)	-0.04 (-1%)	1.03 (37%)	1.04 (38%)	0.01 (+1%)
005: L (%)	1.19 (57%)	1.44 (69%)	0.25 (+12%)	1.18 (56%)	1.22 (58%)	0.04 (+2%)
006: L (%)	1.15 (37%)	1.02 (34%)	-0.13 (-3%)	1.14 (38%)	1.11 (37%)	-0.03 (-1%)
Mean FEV_1 in L (+/- SEM)	**1.10 (± 0.07)**	**1.13 (± 0.16)**	**0.03 (± 0.11)**	**1.07 (± 0.05)**	**1.05 (± 0.08)**	**-0.02 (± 0.03)**
Mean FEV_1 as % of predicted (+/- SEM)	**43 (± 7)**	**46 (± 12)**	**3 (± 5)**	**45 (± 5)**	**44% (± 5)**	**-1 (± 2)**

Note: (See Table 9 footnote regarding dosing history). Spirometry was performed at baseline (B) and day 28 (D28). Differences in FEV_1 (L and % predicted) were calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

Impulse oscillometry system (IOS) measures airway resistance. Resistance to sound waves of different frequencies represents either large airway or total airway resistance. Resistance at 5 Hz is representative of total airway resistance (i.e. R_5), while large airway resistance is represented by resistance to sound waves of 20 Hz (i.e. R_{20}). Small airway resistance can therefore be calculated by subtracting R_{20} from R_5 (R_5 - R_{20}). In COPD, the small airways are the primary site of airflow obstruction due to chronic inflammation and remodelling. This is reflected in an elevated R_5 - R_{20}. The relative effects of solithromycin and placebo on both R_5 and R_5 - R_{20} were therefore evaluated as secondary outcomes, as shown in Table 13 and Table 14. There were no clear trends suggesting that either solithromycin or placebo influenced total or small airway resistance. As before, no statistical comparisons were made.

Table 13 Effect of Placebo and Solithromycin on Total Airway Resistance (R_5)

	Solithromycin Treatment Period IOS, R_5 in kPaL-1.s			Placebo Treatment Period IOS, R_5 in kPaL-1.s		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	0.47	0.52	0.05
004	0.67	0.72	0.05	0.66	0.57	-0.09
005	0.73	0.70	-0.03	0.71	0.82	0.11
006	0.71	0.76	0.05	0.69	0.61	-0.08
Mean	**0.70 (± 0.02)**	**0.73 (± 0.02)**	**0.02 (± 0.03)**	**0.63 (± 0.06)**	**0.63 (± 0.07)**	**0.00 (± 0.05)**

Note: (See Table 9 footnote regarding dosing history). IOS was performed at baseline (B) and day 28 (D28) and measures of R_5 (kPa.L-1.s) were recorded. Differences were then calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

Table 14 Effect of Placebo and Solithromycin on Small Airway Resistance (R_5-R_{20})

	Solithromycin Treatment Period IOS, $R_5 - R_{20}$, in kPaL-1.s			Placebo Treatment Period IOS, $R_5 - R_{20}$, in kPaL-1.s		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	0.04	0.05	0.01
004	0.3	0.38	0.08	0.32	0.24	-0.08
005	0.19	0.18	-0.01	0.23	0.28	0.05
006	0.32	0.36	0.04	0.27	0.27	0
Mean	**0.27 (± 0.04)**	**0.31 (± 0.06)**	**0.04 (± 0.03)**	**0.22 (± 0.06)**	**0.21 (± 0.05)**	**-0.01 (± 0.03)**

Note: (See Table 9 footnote regarding dosing history). IOS was performed at baseline (B) and day 28 (D28) and measures of $R_5 - R_{20}$ (kPa.L-1.s) were recorded. Differences were then calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

The COPD Assessment (CAT) test, an 8-question symptom score, is validated to provide a measure of health status in COPD. Point totals can range from 0 to 40, with scores > 20 suggesting significant disease impact on quality of life. The relative effects of solithromycin and placebo on CAT scores were therefore chosen as a secondary outcome. These data are shown in Table 15, for the subjects who completed 28 days of either treatment. There were no clear trends suggesting an effect on CAT scores. Again, no statistical comparisons were made.

Table 15 Effect of Placebo and Solithromycin on COPD Assessment Test (CAT) Scores

	Solithromycin			Placebo		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	8	8	0
004	12	19	7	18	15	-3
005	30	22	-8	26	28	2
006	9	8	-1	9	7	-2
Mean	**17 (± 7)**	**16 (± 4)**	**-1 (± 4)**	**15 (± 4)**	**15 (± 5)**	**-1 (± 1)**

Note: (See Table 9 footnote regarding dosing history). CATs were undertaken at baseline (B) and day 28 (D28). Differences were then calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

COPD is associated with systemic inflammation and upregulated levels of serum inflammatory markers, such as C-reactive protein (CRP) and fibrinogen. As such, the relative effects of solithromycin and placebo on both CRP and fibrinogen were chosen as secondary outcomes. These data are shown in Table 16 and Table 17, respectively, for the subjects who completed 28 days of either treatment. There were no clear trends suggesting an effect on CRP or fibrinogen. No statistical comparisons were made.

Table 16 Effect of Placebo and Solithromycin on Plasma CRP Levels

	Solithromycin Treatment Period Plasma CRP Levels (mg/mL)			Placebo Treatment Period Plasma CRP Levels (mg/mL)		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	1	8	7
004	3	10	7	1	2	1
005	17	7	-10	16	9	-7
006	4	2	-2	4	2	-2
Mean	**8 (± 5)**	**6 (± 2)**	**-2 (± 5)**	**6 (± 4)**	**5 (± 2)**	**0 (± 3)**

Note: (See Table 9 footnote regarding dosing history). Blood samples were taken at baseline (B) and day 28 (D28). Concentrations of CRP (mg/ml) were reported by the Royal Brompton & Harefield clinical biochemistry laboratory. Differences were then calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

Table 17 Effect of Placebo and Solithromycin on Plasma Fibrinogen Levels

	Solithromycin Treatment Period Plasma Fibrinogen Levels (g/L)			Placebo Treatment Period Plasma Fibrinogen Levels (g/L)		
Subject	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**	**Baseline (B)**	**Day 28 (D28)**	**Δ (D28 - B)**
002	-	-	-	3.8	5.5	1.7
004	3.7	4.2	0.5	2.7	3.6	0.9
005	4.5	4.2	-0.3	4.8	4.3	-0.5
006	3.8	4.3	0.5	4.4	4.2	-0.2
Mean	**4.0 (± 0.3)**	**4.2 (± 0.0)**	**0.2 (±0.3)**	**3.9 (± 0.5)**	**4.4 (± 0.4)**	**0.5 (± 0.5)**

Note: (See Table 9 footnote regarding dosing history). Blood samples were taken at baseline (B) and Day 28 (D28). Concentrations of fibrinogen (g/L) were reported by the Royal Brompton & Harefield clinical haematology laboratory. Differences were then calculated (Δ (D28 - B)). Data presented are individual subjects and means (+/- SEM).

11.3.1 Analysis of Efficacy

The primary and secondary outcome measures are summarised in Figure 1. These data are presented as mean changes in each parameter following 28 days of treatment with either placebo or solithromycin. Due to the small sample size, no statistical comparisons have been made. However, there are trends suggesting that solithromycin (and not placebo) reduces sputum neutrophils and the concentrations of CXCL8 in both sputum and nasal ELF. There were no clear trends suggesting any effect on FEV_1 (L or % predicted), R_5, C-reactive protein, fibrinogen or CAT scores, by either solithromycin or placebo.

Figure 1 Effect of Placebo and Solithromycin on Outcomes

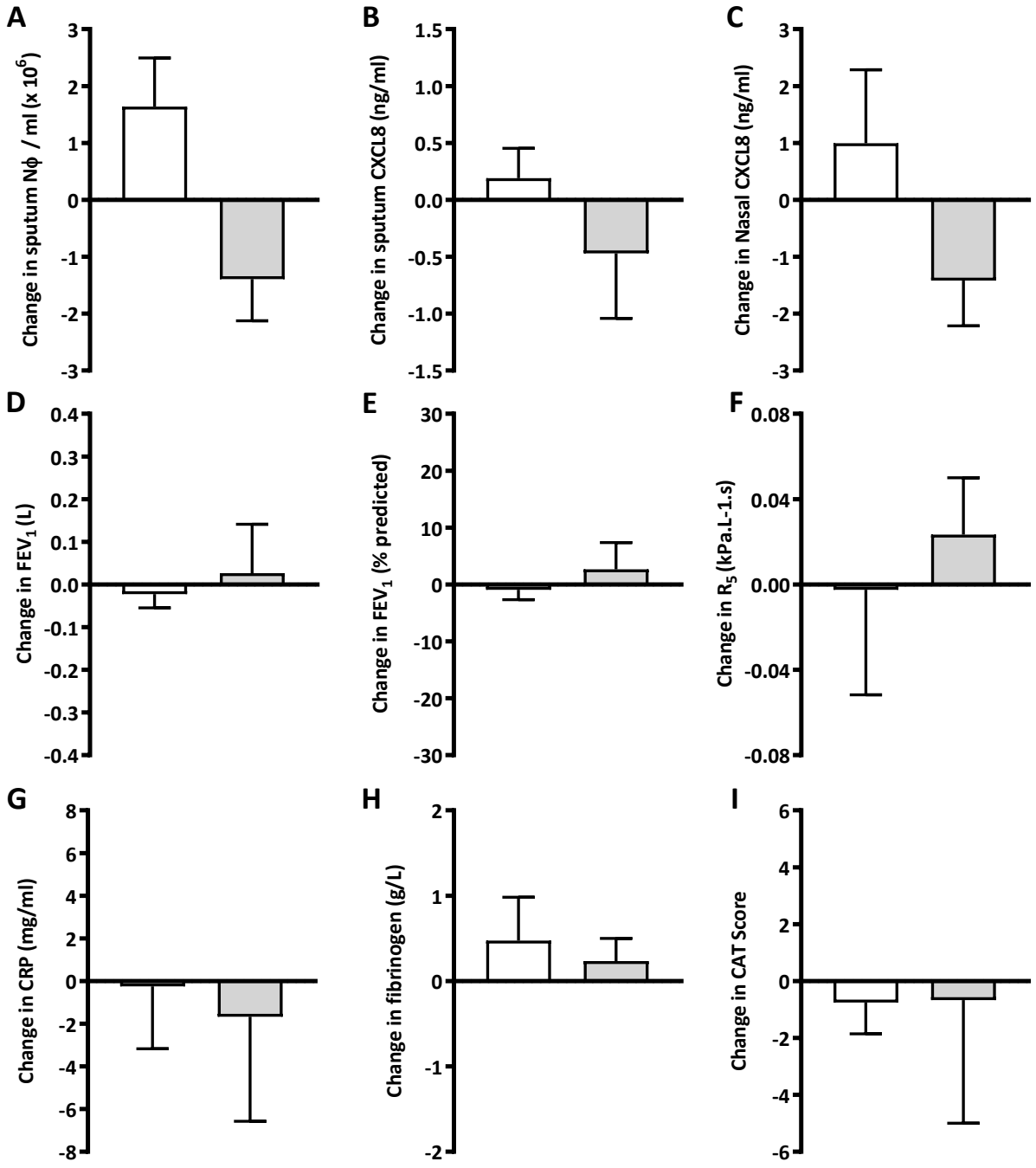

Figure 1 Effect of placebo and solithromycin on selected primary and secondary outcomes: (A) Change in sputum neutrophils (NΦ) / ml (B) Change in concentrations of sputum CXCL8 (C) Change in concentrations of nasal CXCL8 (D) Change in FEV_1 (L) (E) Change in FEV_1 (% predicted) (F) Change in R_5 (G) Change in levels of CRP (H) Change in levels of fibrinogen (I) Change in CAT scores. Subjects received placebo (n = 4) and solithromycin (n = 3) as part of the CE01-204 protocol. Primary and secondary outcome measures were subsequently analysed. Data presented are mean ± SEM for placebo (□) and solithromycin (■).

One subject with eczema reported that this skin condition improved during the 28 day treatment period with solithromycin.

11.3.2 Statistical/Analytical Issues

Due to the low sample size, statistical analyses were not possible.

11.4 Pharmacokinetics

Plasma concentrations of solithromycin (CEM-101) were measured from all subjects approximately 4 hours post dose on Days 7, 14, and 28 (±2 Days) of each Treatment Period. Plasma concentrations of solithromycin (CEM-101) and the two major metabolites, N-Acetyl-CEM-102 and CEM-214 demonstrated considerable interindividual and intraindividual variability. CEM-101 concentrations did not appear to accumulate over the 4 weeks of treatment with daily doses of 400 mg. Individual plasma concentration data are listed in Table 18.

Table 18 Solithromycin Individual Subject Plasma Concentrations

Analyte	Treatment Period	Day	Time	Subject I.D.				
				001	002	004	005	006
CEM-101	1	7	4 h	718	BQL	BQL	BQL	840
(ng/mL)		14	4 h	993	BQL	BQL	BQL	797
		23	Unscheduled	750	-	-	-	802
		28	4 h	-	BQL	BQL	BQL	447
		31	Unscheduled	-	-	-	BQL	-
	2	7	4 h	-	-	271	1,200	BQL
		14	4 h	-	-	180	832	BQL
		28	4 h	-	-	321	692	BQL
N-acetyl CEM-101	1	7	4 h	45.1	BQL	BQL	BQL	44.2
(ng/mL)		14	4 h	90.9	BQL	BQL	BQL	53.1
		23	Unscheduled	112	-	-	-	39.1
		28	4 h	-	BQL	BQL	BQL	22.5
		31	Unscheduled	-	-	-	BQL	-
	2	7	4 h	-	-	26.6	33.7	BQL
		14	4 h	-	-	15.0	28.3	BQL
		28	4 h	-	-	38.6	22.2	BQL
CEM-214	1	7	4 h	32.3	BQL	BQL	BQL	20.5
(ng/mL)		14	4 h	22.8	BQL	BQL	BQL	23.1
		28	4 h	-	BQL	BQL	BQL	17.0
		31	Unscheduled	-	-	-	BQL	-
			Unscheduled	18.7	-	-	-	27.7
	2	7	4 h	-	-	13.1	30.7	BQL
		14	4 h	-	-	6.67	31.2	BQL
		28	4 h	-	-	11.3	29.5	BQL

11.4.1 Drug Dose, Drug Concentration, and Relationships to Response

Too few data were collected to allow an analysis.

11.4.2 Drug-Drug and Drug-Disease Interactions

No proven drug-drug and drug-disease interactions were demonstrated. However, it remains possible that finasteride and a statin may have contributed to the hepatoxic adverse events experienced by two separate subjects (see below). It is also possible that the high incidence of hepatotoxicity demonstrated in CE01-204 (not observed previously) was related to its use in the COPD population. This is judged to be very unlikely.

11.5 Efficacy Conclusions

No conclusions can be made as too few data were available for statistical analysis.

12 SAFETY EVALUATION

12.1 Extent of Exposure

A total of 4 subjects were exposed to solithromycin (Subjects 001, 004, 005 and 006). Subject 001 was withdrawn before receiving 28 days of treatment and was not included in the efficacy analysis but has been included in the safety evaluation. Subjects 004 and 006 received the full 28 days of treatment, and Subject 005 stopped dosing on Day 26 as a matter of convenience; all are included in both the efficacy and safety evaluations.

12.2 Adverse Events

The most important observations were related to hepatic safety of solithromycin dosing. Three subjects developed ALT elevations to greater than 3-fold the ULN, (Subjects 001, 005 and 006), with onset at Day 15 in two subjects (in one case resolving with ongoing solithromycin dosing), and at Day 26 in the third subject. In Subject 001, as described below, this was a component of an episode of cholestatic hepatitis, characterized by elevation of ALT, AST, alkaline phosphatase and bilirubin. This event was reported as a SUSAR. Other adverse events are listed below.

12.2.1 Brief Summary of Adverse Events

Adverse event data were collected from the time informed consent was obtained through discharge from the study. All clearly related signs, symptoms, and results of diagnostic procedures performed because of an AE were grouped together and recorded as a single diagnosis. Laboratory abnormalities were not considered AEs unless they were associated with clinical signs or symptoms, or required medical intervention. If the AE was a laboratory abnormality that was part of a clinical condition or syndrome, it was recorded as the syndrome or diagnosis rather than the individual laboratory abnormality.

From the time of enrollment through the end of the study, 5 (83%) of the 6 randomized subjects reported 7 AEs. In the Safety Population which is limited to subjects who received study drug (either solithromycin or placebo), 3 subjects reported 4 treatment emergent adverse events (TEAEs) during the solithromycin treatment period and 1 subject reported 1 TEAE during the placebo treatment period. (Table 19). Most TEAEs were of mild or moderate intensity (Section 12.2.3.1) and only 1 event was severe in intensity. The most common TEAEs (Table 20) were Gastrointestinal disorders in 4 subjects and Hepatobilary disorders in one subject.

There were no deaths or pregnancies reported. One serious adverse event of elevated liver tests was reported and the subject discontinued study drug on Day 24 (Section 12.2.3.1).

12.2.2 Display of Adverse Events

The frequency of treatment emergent AEs (TEAEs), treatment related TEAEs, AE severity, SAEs, and AEs leading to study drug discontinuation or withdrawl from study are presented in Table 19.

Table 19 Summary of Treatment Emergent Adverse Event (TEAEs)

	Overall N=5 n (%)	Placebo N=4 n (%)	Solithromycin N=4 n (%)
Subjects with ≥ 1 TEAE	4 (80%)	1 (25%)	3 (75%)
Subjects with ≥ 1 treatment-related TEAE	3 (60%)	0 (0%)	3 (75%)
Subjects with ≥ 1 severe TEAE	1 (20%)	0 (0%)	1(25%)
Subjects with ≥ 1 SAE	1 (20%)	0 (0%)	1 (25%)
Subjects with ≥ 1 TEAE resulting in premature discontinuation of study drug	1 (20%)	0 (0%)	1 (25%)
Subjects with ≥ 1 TEAE resulting in premature withdrawal from study	1 (20%)	0 (0%)	1 (25%)

12.2.3 Analysis of Adverse Events

Each adverse event was analysed case by case. As expected, gastrointestinal side-effects were reported by subjects receiving solithromycin. The magnitude of the liver parameter disturbance was unexpected and in the case of Subject 001, was severe and reported to the MHRA and REC as a SUSAR. This ultimately led to the early termination of CE01-204.

12.2.3.1 All Adverse Events

Table 20 lists all reported treatment emergent adverse events.

Table 20 Listing of All Treatment Emergent Adverse Event

System Organ Class AE Verbatim Term	Overall N=5 n (%)	Placebo N=4 n (%)	Solithromycin 400 mg N=4 n (%)
Gastrointestinal disorders	4 (80%)	1 (25%)	3 (75%)
Abdominal Discomfort/Pain	3 (75%)	1 (25%)	2 (50%)
Nausea	1 (25%)	1 (0%)	0 (0%)
Flatulance	1 (25%)	0 (0%)	1 (25%)
Epigastric Discomfort	1 (25%)	0 (0%)	1 (25%)
Gastro-oesophageal reflux	2 (50%)	0 (0%)	2 (50%)
Hepatobilary disorders	1 (20%)	0 (0%)	1 (25%)
Derranged LFTs	1 (20%)	0 (0%)	1 (20%)
Skin and subcutaneous disorders	1 (20%)	1 (20%)	0 (0%)
Puffy eyes	1 (20%)	1 (20%)	0 (0%)

Adverse events are briefly summarized, by subject. In addition to liver safety signals (either reported as an AE, or identified as a laboratory abnormality without symptoms), the most commonly observed adverse effects with solithromycin were gastrointestinal. An episode of peripheral eosinophilia was observed in Subject 001, which was considered a component of the

cholestatic hepatitis episode and not reported as a unique AE. No cardiac, renal or neurological AEs were reported.

Subject 001:

- 48 h of mild self-resolving abdominal pain was reported on Day 9 of solithromycin treatment. Thought to "possibly" be related to solithromycin treatment. Classed as a non-serious AE.
- Significant cholestatic hepatitis with eosinophilia developed from Day 15 of solithromycin treatment, and was considered study drug related. This was classified as an SAE and reported as a SUSAR. See narrative below (Section 12.3.4)

Subject 002:

- "Puffy" eyes reported by subject during placebo treatment. Self-resolving and not evident on assessment at the next study visit.
- Nausea, wretching and more purulent sputum. This occurred while the subject was off all study medication and after completing treatment with placebo, prior to starting solithromycin (i.e. during washout). These symptoms were attributed to a COPD exacerbation (CRP raised at 92 mg/L). The subject was therefore withdrawn, due to this exacerbation.

Subject 003:

- No adverse events were recorded.

Subject 004:

- No adverse events were recorded

Subject 005:

- Episode of mild abdominal pain and nausea was reported on Day 9 of solithromycin treatment. Thought to "possibly" be related to solithromycin treatment. Classed as a non-serious AE.
- Seven days of mild abdominal pain, flatulence and gastro-oesophageal reflux (reported on Day 26 of solithromycin treatment). Thought to "possibly" be related to solithromycin treatment. Classed as a non-serioius AE.

Subject 006:

- Single episode of mild epigastric discomfort and gastro-oesophageal reflux (reported on Day 9 of solithromycin treatment). Thought to "possibly" be related to solithromycin treatment. Classed as a non-serioius AE.

12.3 Deaths, Other Serious Adverse Events and Certain Other Significant Adverse Events

12.3.1 Deaths

There were no deaths during the CE01-204 clinical trial.

12.3.2 Other Serious Adverse Events

One subject (001) experienced a serious adverse event (SAE) of cholestatic hepatitis with pruritus. This event was reported as an IND Safety Report (IND 101317: initial report December 24, 2015 [SN0181]; follow-up #1 January 20, 2016 [SN0183]; follow-up #2 February 5, 2016 [SN0187]; and follow-up #3 March 11, 2016 [SN6000]). This same subject (001) had dosing with study drug (solithromycin) discontinued early (Day 23) due to this event. Subject narratives and lab results for this subject are presented in Section 12.3.4.

12.3.3 Other Significant Adverse Events

Two additional subjects (005 and 006) receiving solithromycin experienced ALT elevation to >3×ULN. In subject (006), an asymptomatic elevation of ALT resolved with continued study drug dosing. In another subject (005), ALT elevation was noted at the end of treatment, and monitored during its resolution. Subject narratives and lab summaries for each of these subjects are presented in Section 12.3.4.

12.3.4 Narratives of Deaths, Other Serious Adverse Events, and Other Significant Adverse Events

Subject 001: This 69-year-old male with a history of COPD and benign prostatic hypertrophy received 400 mg oral solithromycin for 23 days (of a planned 28 day course). Concomitant medications at the time of enrollment included fluticasone/salmeterol and salbutamol metered-dose inhalers and oral finasteride 5 mg QD.

At baseline and Day 9, all hepatic safety tests were in the normal range. On Day 15, ALT was 95 U/L (1.4×ULN), AST 106 U/L (2.6×ULN), ALP 277 U/L (2.1×ULN), and total bilirubin 0.8 mg/dL. On Day 23, further increases in ALT to 476 U/L (11.9×ULN), AST to 368 U/L (9.0×ULN) and ALP to 1316 U/L (10.1×ULN) were noted, as well as elevation of total bilirubin to 4.0 mg/dL (3.4×ULN) with direct bilirubin 2.2 mg/dL (3.6×ULN) and eosinophil count 1.6×10^9/L (3.2×ULN). Administration of solithromycin and finasteride was discontinued (last dose Day 23).

The subject was mildly icteric, with pruritus. An ultrasound of the liver and biliary tract was normal, and viral hepatitis screens were negative. One day later on Day 24, improvement was evident. The subject was monitored for an additional 4 weeks and the results continued to improve with only ALP remaining 1.3×ULN at the final follow-up visit. The subject did not require hospitalization for this event. Liver function as measured by prothrombin time (INR) remained normal throughout (either 0.9 or 1 at all timepoints evaluated – Days 15, 23, 24, and 34).

Plasma concentrations of solithromycin and finasteride were measured on Day 9, Day 15 and Day 23 at about 4 hours post dose (predicted T_{max}). Solithromycin concentrations were 718, 993 and 750 ng/mL, within the expected range. Finasteride concentrations were elevated 3 to 4-fold above the reported values for a 5 mg dose.

The concomitant elevation of alkaline phosphatase (confirmed by elevations of GGT), ALT and bilirubin characterized this event as an episode of cholestatic hepatitis. Upon discontinuation of both solithromycin and finasteride the laboratory abnormalities resolved, and no clinically significant events related to the LFT elevation were reported; Table 21.

Table 21 Subject 001 Hepatic Safety Parameters

	ALT		AST		Bilirubin		ALP					
Visit/ Day	U/L	×ULN	U/L	×ULN	Total ULN:1.2 mg/dL	Direct ULN:0.4 mg/dL	U/L	×ULN	WBC ×10³/µL	EOS ×10³/µL	Creat mg/dL	PT INR
Baseline	20	0.5	29	0.7	0.7	0.2	78	0.6	5.7	0.3	0.8	
Day 9	32	0.8	34	0.8	0.8	0.2	74	0.6	7.1	0.2	0.8	
Day 15	95	1.4	106	2.6	0.8	0.3	277	2.1	6.3	0.4	0.9	0.9
Day 23	476	11.9	368	9.0	4	2.2	1316	10.1	9.5	1.6	0.8	0.9
Last dose of solithromycin received on Day 23												
Day 24	427	10.7	322	7.9	2.9	1.5	1155	8.9	9.2	1.8	0.7	1
Day 28	269	6.7	144	3.5	1.2	0.5	969	7.5	6.8	1.2	0.7	-
Day 34	92	2.3	59	1.4	0.8	-	471	3.6	6.1	0.7	0.7	0.9
Day 50	27	-	22	-	0.5	0.2	177	1.4	7.2	0.4	0.7	-
Day 64	18	-	22	-	0.6	0.2	118	-	5.7	0.3	0.7	-

Subject 005: This 65-year-old female with a history of COPD, hypothyroidism, hyperlipidemia and coronary artery disease received 400 mg oral solithromycin daily for 26 days (of a planned 28 day course). Concomitant medications included triotropium, atorvastatin (20 mg QD), levothyroxine, seretide and salbutamol metered-dose inhaler. Following the end of her planned 28 day solithromycin dosing period (truncated to 26 days due to clinic schedules), the subject developed an asymptomatic elevation of ALT (to 7.3×ULN) (observed on Day 31) which recovered quickly, and was not associated with bilirubin elevation.

At baseline and through Day 15, all hepatic safety tests were within normal ranges Table 22. On Day 26, ALT was 141 U/L (3.5×ULN), AST 89 U/L (2.2×ULN), ALP 128 U/L, and total bilirubin 0.5 mg/dL. Dosing was stopped based on site schedules with the Day 26 dose. On Day 31, ALT was 292 U/L (7.3×ULN), AST 126 U/L (3.1×ULN) and ALP 190 U/L (1.5×ULN. On Day 36, values improved. After Day 37, the subject refused further evaluation. Total and direct bilirubin remained normal at all visits and she remained completely asymptomatic throughout this episode. No peripheral eosinophilia was observed.

Plasma samples for assays of solithromycin and atorvastatin concentrations were collected at steady state 4h after the solithromycin dose at the 1 week visit in both the solithromycin and placebo treatment periods. The solithromycin concentrations were 1200 ng/mL and undetectable,

respectively, and the results for atorvastatin were 6.32 and 2.53 ng/ml, respectively. On Day 26, when the ALT was increased, the solithromycin concentration was 692 ng/mL and the atorvastatin concentration was 4.25 ng/mL. These values are within the anticipated ranges for the administered doses of the two drugs. Creatine phosphokinase, a marker of statin toxicity, was measured during the study and the values remained between 32 and 47 U/L (ULN = 171 U/L).

Table 22 Subject 005 Hepatic Safety Parameters

Visit/ Day	ALT		AST		Bilirubin		ALP		WBC	EOS	Creat	PT
	ULN:40		ULN:41		Total ULN:1.2	Direct ULN:0.4	ULN:130					
	U/L	×ULN	U/L	×ULN	mg/dL	mg/dL	U/L	×ULN	×10³/µL	×10³/µL	mg/dL	INR
Baseline	24	0.6	27	0.7	0.5	0.1	133	1.0	7.4	0.2	0.8	
Day 9	24	0.6	25	0,6	0.4	0.1	137	1.1	7.3	0.2	0.7	1.0
Day 15	29	0.7	27	0.7	0.4	0.1	139	1.1	8.8	0.2	0.6	1.0
Day 26	141	3.5	89	2.2	0.5	0.1	128	1.0	8.5	0.4	0.8	1.0
Last dose of solithromycin received on Day 26												
Day 31	292	7.3	126	3.1	0.4	0.1	190	1.5	6.7	0.3	0.7	0.9
Day 36	100	2.5	37	-	0.4	0.1	171	1.3	-	0.3	0.7	-
Day 37	78	-	n/a	-	0.6	-	168	-	-	-	-	-

Subject 006: This 73-year-old male with a history of COPD received oral solithromycin, 400 mg QD, for 28 days. He was treated for his other medical conditions with tiotropium, Seretide and salbutamol. On Day 15, elevation of ALT to 165 U/L (4.1×ULN) was noted, with parallel AST elevation to 2.7×ULN and ALP elevation to 1.3×ULN (Table 23). Bilirubin was not elevated, and the subject was asymptomatic. Study drug dosing continued, and on repeat evaluation at Day 23, ALT, AST, and ALP had improved significantly. On Day 28, the last day of study drug dosing, ALT, AST, and ALP were all normal. Solithromycin concentrations on Days 8, 14 and 26 at 4 hours post drug administration were 840, 797 and 802 ng/mL, respectively, within the range of expected values for the 400 mg oral QD dose. No peripheral eosinophilia was observed.

Table 23 Subject 006 Hepatic Safety Parameters

Visit/ Day	ALT		AST		Bilirubin		ALP		EOS	Creat
	ULN:40		ULN:41		Total ULN:1.2	Direct ULN:0.4	ULN:130			
	U/L	×ULN	U/L	×ULN	mg/dL	mg/dL	U/L	×ULN	×10³/µL	mg/dL
Baseline	17		21		0.6	0.1	109		0.1	0.9
Day 9	17		20		0.7	0.1	99		0.2	0.9
Day 15	165	4.1	108	2.7	0.8	0.2	174	1.3	0.4	1.0
Day 23	53	1.3	26		0.5	0.1	144	1.1	0.2	0.9
Day 28	30		25		0.5	0.1	129		0.2	1.0
Last dose of solithromycin received on Day 28										

12.3.5 Discussion of Deaths, Other Serious Adverse Events and Other Significant Adverse Events

The observed gastrointestinal adverse events of abdominal discomfort or pain, flautlance, reflux and epigastric discomfort among patients receiving solithromycin were self-limited and of mild severity. Gastrointestinal adverse events are not unexpected with solithromycin, or with macrolide antibiotics in general. These events were not considered treatment limiting. Their observation in 75% of patients is not expected, but this is the first trial in which subjects received solithromycin for this duration of time, allowing more time for AEs to accrue.

The Serious Adverse Event of cholestatic hepatitis was not expected, and was reported as a SUSAR to MHRA (and as an expedited SAE report to FDA). Upon recognition of the event, study drug dosing was discontinued (on Day 23). It is evident, however, that the episode had its start between Day 9 and Day 15 of study drug adminstration, given that ALT was elevated (to 1.4xULN) on the Day 15 visit (and had been normal on Day 9. Following cessation of dosing, the laboratory parameters and clinical signs recovered relatively rapidly (by Day 34, bilirubin was normal, and by Day 50, ALT was normal).

The observation of cholestatic hepatitis in Subject 001, and the additional observation of ALT elevation to peak values 7.3 fold and 4.1 fold ULN in two additional patients, respectively, made clear that daily oral dosing with 400 mg of solithromycin for greater than 9 to 14 days has an unacceptable safety profile. On the basis of these observations, the study was terminated.

12.4 Clinical Laboratory Evaluation

Laboratory values were recorded according to the CE01-204 for all subjects during the trial period. Apart from the previously identified changes in liver function and eosinophilia, no other clinically meaningful haematological or biochemical abnormalities were detected while subjects were receiving solithromycin.

12.5 Vital Signs, Physical Findings, and Other Observations Related to Safety

There were no other identified changes (trends) in vital signs, physical findings or other observations that were related to the use of solithromcin. As already identified, Subject 1 developed icterus and pruritus in association with the cholestatic hepatitis.

12.6 Safety Conclusions

At a dose of 400 mg orally (once daily over 28 days) solithromycin is associated with an unacceptable risk of significant hepatotoxicity.

13 DISCUSSION AND OVERALL CONCLUSIONS

The trial was terminated early due to the observation of cholestatic hepatitis in a single subject, and marked ALT elevation in two others, during or following solithromycin dosing. Data from the four subjects taking solithromycin suggested a trend towards reduced sputum neutrophil numbers and CXCL8 levels; however, the data set is too small for analyses.

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