Supplementary Online Content


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References

This supplementary material has been provided by the authors to give readers additional information about their work.
eMethods

Inclusion criteria
1. Able to provide written informed consent.
2. Confirmed diagnosis of bronchiectasis by HRCT within 3 years.
3. Airways obstruction on spirometry (ratio FEV₁/FVC <0.7) and FEV₁ ≥25% predicted 
4. Chronic productive cough with at least 5 mLs sputum production per day.
5. At least two (2) exacerbations of bronchiectasis requiring either oral or intravenous supplemental antibiotic therapy (of at least 7 days on each occasion) in the prior 12 months.
7. Clinically stable for at least four weeks (defined as no symptoms of exacerbation, no requirement for supplemental antibiotic therapy, and FEV₁ within 10% of best recently recorded value where available).

Exclusion criteria
1. Bronchiectasis as a result of CF or focal endobronchial obstruction.
2. Currently active tuberculosis or non-tuberculous mycobacterial (NTM) infection. Subjects with evidence of prior pulmonary NTM infection could be included only if they have completed a course of therapy that is deemed successful on the basis of negative NTM cultures following cessation of therapy. All subjects required a negative NTM culture prior to screening.
3. Any symptoms or signs to suggest recent deterioration in respiratory disease, including exacerbation of pulmonary disease (as previously defined) in the preceding 4 weeks.
4. Any change to medications in the preceding 4 weeks.
5. Prescription of either oral or intravenous antibiotic therapy in the preceding 4 weeks.
6. Cigarette smoking within the preceding 6 months.
7. Any history of malignant arrhythmia (unless in the immediate post-myocardial infarction period and not requiring any regular therapy) or QTc prolongation (> 440 ms) on baseline ECG.
8. Any of the following within the three (3) months prior to enrolment:
   - Acute MI
   - Acute CVA
   - Major surgery
9. History of any of the following:
   - Active malignancy (excepting non-melanoma skin malignancies that have been treated and considered cured)
   - Listed for transplantation
   - Any other significant active illness likely to affect the patient’s survival within 12 months
   - Receiving long-term domiciliary oxygen therapy
10. Allergy to macrolide antibiotics, other than minor, dose-related gastrointestinal intolerance that would not be anticipated to recur with low-dose erythromycin.
11. Any prescription or receipt of long-term macrolide antibiotics, or receipt of a treatment course within 4 weeks.
12. Predominant diagnosis of emphysema (rather than bronchiectasis) on HRCT scan of the chest.
13. Requirement for supplemental oxygen therapy.
14. Inability to complete required study procedures for whatever reason (including 6 minute walk test, hypertonic saline sputum induction).
15. Respiratory symptoms (including cough, sputum production, recurrent exacerbations) not predominantly the result of bronchiectasis in the opinion of the PI; where treatable causes for exacerbations existed, these were treated before considering trial enrolment.

Excluded medications
1. Macrolide antibiotics – long-term macrolide use was an absolute exclusion, however subjects who had received a short duration (less than 6 weeks) treatment course were eligible provided they had at least 4 weeks washout.
2. Long term oral antibiotic administration for infection prophylaxis (eg doxycycline).
3. Any other intravenous or oral antibiotic within 4 weeks.
4. While erythromycin in the current study was administered in a low dose, possible drug interactions in all patients entering the study were considered. Subjects using the following medications were not eligible for the study:
   - ergotamine or dihydroergotamine
   - triazolam/ alprazolam
   - sildenafil

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• azole antifungals (ketoconazole, itraconazole, fluconazole)
• disopyramide
• quinidine

Concomitant HMG-CoA reductase inhibitor (‘statin’) use was permitted, however subjects on high-dose statins (equivalent to 80 mgs daily of simvastatin) required dose reduction by half at study entry.

Subjects prescribed diltiazem or verapamil at study entry required careful ECG and clinical screening at enrolment and at all followup visits to ensure no evidence of clinically relevant increases in levels of these medications (specifically with respect to pulse rate and evidence of any degree of heart block)
- if these subjects were bradycardic (resting PR< 56) or had ECG evidence of heart block, they were not eligible for the study (unless dose reduction was indicated)

The following medications were permitted, provided they had been a regular medication for at least 6 months (with the requisite number of exacerbations whilst on this therapy):
1. Inhaled antibiotics in chronic, daily, stable dose.
2. Inhaled mucolytic therapies (hypertonic saline, mannitol, domnast alpha, N-acetylcysteine).
3. Oral mucolytics or expectorants.

### Procedures

*Leicester Cough Questionnaire¹ - Applied to visit 1-9 inclusive*

*St George Respiratory Questionnaire ² - Applied to Visit 1-9 Inclusive*

*24 hour Sputum Collection - Applied to visits 1 – 9 inclusive*

The subject was given a sterile jar to collect the sputum in. The collection commenced upon rising in the morning and was completed 24 hours later (eg 8am until 8am). All subjects were asked to undertake morning and evening physiotherapy using their usual method of chest clearance technique. Subjects were instructed to collect ALL sputum produced spontaneously or after coughing over a single daytime 24 hour period. It was important that the sample came from the lungs and was not salivary. Subjects were encouraged not to swallow sputum, but to collect. Each 24 hour collection period was as similar as possible in terms of physiotherapy regimens. When the sample was weighed, the weight of the empty jar was subtracted from the final weight. The same set of scales was used for weighing all study samples and the scales zeroed before each measure. These samples were not used for any other analyses including microbiology.

*Haematology, Liver Function, CRP - Applied to Visits 1, 8*

*6 Minute Walk Test - Applied to Visits 1, 5, 8*

*Electrocardiogram - Applied to Visits 1, 2, 5, 8*

*Sputum microbiology – Applied to Visits 1, 5, 8*

Sputum processing for culture and sensitivity testing was performed in the Division of Microbiology, Mater Pathology. Sputum was transported to the laboratory within 60 min of collection and processed within 3 h (refrigerated at 4°C in the interim). The most purulent portion of the specimen was selected and streaked directly onto horse blood agar (HBA), MacConkey agar, chocolate agar supplemented with bacitracin (CHOC-B), Sabouraud agar, and mannitol salt agar. Plates were incubated at 37°C (HBA and CHOC-B in CO2 and CHOC-B anaerobically and the rest in O2) for at least 48 h and examined daily.

*Sputum inflammatory cell counts – Applied to Visits 1, 5, 8*

Collected sputum was placed on ice immediately and transferred for processing within 60 minutes. Sputum was processed according to the methods of the US Cystic Fibrosis Therapeutics Development Network Standard Operating Procedure.² Briefly, an equal volume of sterile 10% dithiothreitol (DTT) (Sputolysin; Calbiochem-Novabiochem Corp., San Diego, CA), was added to the sputum, then incubated in a shaking water bath at 37°C for 5-10 min, and gently mixed using a transfer pipette at 5-min intervals. A further three times the volume of both DTT and phosphate-buffered saline (Dulbecco’s; Gibco BRL, Grand Island, NY) was added and the mixture incubated again in the 37°C shaking water bath for another 5-10 min. Ten microliters of the homogenized sputum samples, mixed with Trypan Blue, was used to calculate total cell counts, using a standard hemacytometer. A further 0.25-0.50 ml of both samples was used to prepare cytospin slides for differential cell counts. After staining the slides with Wright’s stain, 300 cells were counted and cell differentials calculated.

*Throat swabs for oropharyngeal streptococcal culture and sensitivity testing – Applied to Visits 1, 2, 8*

This was performed using a modification of the methods described by Malhotra-Kumar et al.³ Samples of oropharyngeal flora were obtained by means of a swab pressed over the tonsils and posterior pharyngeal wall, avoiding jaws, teeth and gingiva on withdrawal. Swabs were collected at visits 1, 2 and 8 and placed in an
aerobic medium containing skimmed milk, glucose and glycerol adapted from Gibson and Khoury\(^5\) and stored at -80°C until further analyses. The samples were then thawed, vortexed and inoculated on Columbia CNA agar (Beckton Dickinson) with or without erythromycin (2µg/mL) using a sterile spreader. Specific dilutions were performed and the colony count from the lowest dilution plate that contained discrete streptococcal colonies was recorded.

Plates were incubated overnight at 37°C in 5% CO\(_2\)/95% air. Streptococcal densities were determined by counting colonies on the appropriate dilution plate, normalised for the inoculated sample volume and dilution factor. The proportion of macrolide-resistant streptococci was determined by division of the [normalised] number of colonies on the erythromycin containing plates by the number of colonies on plates without erythromycin.

Media quality control was performed to ensure growth of gram positive organisms only and to confirm growth of only erythromycin resistant strains on erythromycin containing agar. Sterility testing was performed; all plates tested remained sterile at room temperature and 37°C during 48 hours incubation.

**Protocol-defined pulmonary exacerbations (PDPE’s):**

(Applied to all visits, including telephone visits, and to all episodes of contact)

Using a modification of the Anthonisen criteria,\(^6\) a subject was considered to have a pulmonary exacerbation when they had persistent (> 24 hour) increase in sputum volume or purulence in addition to deterioration in at least 2 other, different of the following symptoms: sputum volume, sputum purulence, cough, dyspnoea, chest pain, new haemoptysis (to meet criteria required 3 separate symptoms overall; ie if a subject described increase in sputum volume, then this symptom could not also be counted as one of the ‘additional’ symptoms, although increased sputum purulence could be counted as a second, separate symptom if present).

Put in a different way, PDPE required deteriorations in at least 3 of the following, with at least 1 of the major criteria:

- Major criteria: increase in sputum volume or sputum purulence.
- Minor criteria: cough, dyspnoea, chest pain or haemoptysis.

(ie PDPE= 1 major and 2 minor or 2 major and 1 minor)

In order to be counted as separate PDPE’s, sequential episodes required unequivocal resolution of symptoms from the first event AND >14 days from the end of one event to the commencement of the subsequent event. If both criteria were not met, the exacerbation was counted as a single, continuing event.

Subjects were provided with 24 hour contact details and directed to contact study staff (MLM or DJS) at any time (including after hours) should they develop either symptoms of exacerbation or feel that they required antibiotic therapy. Subjects were directed to ensure that antibiotic prescription was provided by and directed through study staff rather than alternative sources. Criteria for PDPE were adjudicated by DJS and MLM.

Criteria for exacerbation were adjudicated by telephone if subjects contacted study staff outside trial visits, and in those meeting criteria for PDPE a prescription was faxed to the subject’s local pharmacy for collection.

Subjects were not routinely reviewed at the study centre in such circumstances, unless symptoms were inconsistent with an exacerbation, the subjects was too unwell to be managed as an outpatient or if symptoms failed to subsequently respond appropriately to therapy. Antibiotic prescriptions were directed by DJS, provided by study staff and standardized according to microbiology and antibiotic tolerance.

**Antibiotic selection for PDPE’s:**

All antibiotic prescriptions were directed by one study member (DJS), were provided by study staff and were standardised according to the results of the most recent respiratory microbiology sample and subject antibiotic tolerance. Oral antibiotic selection was based upon the most recent microbiology result, according to the following guide (subjects were instructed to cease antibiotics once symptoms of exacerbation resolved, even if antibiotic supply remained; microorganisms not discussed below had antibiotic selection determined according to the results of antimicrobial susceptibility testing):

- ‘Normal flora’/ no respiratory pathogens isolated – oral amoxycillin 500 mg/ clavulanic acid 125 mg, one tablet twice daily for 10 days
  - If penicillin allergic (simple), cephalexin 500 mg, one capsule four times daily for 10 days
  - If penicillin anaphylaxis, antibiotic selection individualised by DJS according to results of prior airway microbiology, prior antibiotic history and tolerability, etc

*P. aeruginosa* – oral ciprofloxacin 250-750 mg (generally 500 mg) twice daily for 14 days
*H. influenzae* – either oral amoxicillin 500 mg thrice daily for 7-14 days or oral amoxicillin 500 mg/ clavulanic acid 125 mg, one tablet twice daily for 10 days according to results of antimicrobial susceptibility testing
- if penicillin allergic, oral cotrimoxazole (sulphamethoxazole 800 mg/ trimethoprim 160 mg) one tablet twice daily for 10 days

If subjects had persistent symptoms of exacerbation at the completion of the course of antibiotics, repeat respiratory microbiology was obtained and the following antibiotics continued until microbiology results were available:

‘Normal flora’ or *H. influenzae* – same antibiotics as original course, for 5 to 10 days; in the event that symptoms persisted following this further course, oral ciprofloxacin 250-750 mg (generally 500 mg) was commenced twice daily for 14 days for suspected occult gram negative airway infection

*P. aeruginosa* – oral ciprofloxacin was continued and oral cotrimoxazole (sulphamethoxazole 800 mg/ trimethoprim 160 mg) twice daily added, for a further 10 to 14 days
**eResults**

**Pulmonary exacerbations** (see Table E1 for summarised event rates)

Per protocol analysis of the primary outcome measure demonstrated a similar, significant effect of erythromycin upon PDPE’s, although the strength of the effect was stronger than the primary ITT analysis (IRR 0.56, 95% CI 0.41 to 0.76, p=0.001).

All of the remaining results relate to the ITT population:

When considering all pulmonary events for which antibiotics were taken by the subject (ie both PDPE and non-protocol defined PE’s - in these latter circumstances subjects had obtained antibiotics from prescribers outside the trial or from existing antibiotic ‘stores’ in spite of not meeting criteria for PDPE), the effect of erythromycin upon number of ‘exacerbations’ remained significant (total 96 vs 127; mean event rate 1.63 (1.20-2.06) vs 2.19 (1.67-2.71) per patient-year, IRR 0.69, 0.52 to 0.90, p=0.02). Episodes in which subjects requested antibiotics for symptoms that they believed to be indicative of exacerbation, but which did not achieve criteria for PDPE (and antibiotics were neither prescribed nor taken), occurred in 49 cases (mean 0.84, 95% CI 0.5 to 1.19) in the placebo group and 15 (0.25, 95% CI 0.03 to 0.47) in the erythromycin group (difference 0.59, 95% CI 0.19 to 0.99, p=0.005). Adding these episodes to all pulmonary events (ie including all respiratory ‘events’ across the study, whether protocol-defined or not and whether antibiotics were taken or not) confirmed significantly fewer overall respiratory events in the erythromycin arm (111 vs 176; mean rates per patient-year 1.88 (1.37-2.39) vs 3.03 (2.47-3.6), IRR 0.58, 0.46 to 0.74, p=0.0004). Total days of antibiotics, per patient, for all pulmonary events over the 48 weeks trended to a significant effect of erythromycin (median 10 (5 to 20) vs 20 (10 to 25) days, difference 5 (0-14), P=0.12).

Additional exploratory subgroup analyses on pulmonary exacerbations:

Additional post-hoc, exploratory analyses were performed. Subgroup analyses according to degree of sputum neutrophilia and *H.influenzae* infection showed no evidence of subgroup benefit. The subgroup with very high baseline exacerbation rate (≥5 exacerbations in the preceding year) showed a significant benefit with erythromycin.

The subgroup of subjects reporting ≥5 exacerbations at baseline demonstrated a significant response to erythromycin (33 PDPE’s in 22 erythromycin subjects vs 61 in 20 placebo subjects, mean 1.5 (0.85-2.15) vs 3.05 (1.98-4.12), difference 1.55 (0.36-2.74), p=0.01). Test of interaction achieved borderline significance (mean treatment effect per patient-year of erythromycin in subjects with ≥5 exacerbations 1.55 vs subjects with 2-4 exacerbations 0.23 (-0.42 to 0.89), difference 1.32 (95% CI -0.02 to 2.66), p=0.053). Combining the 2 subgroups in whom benefits were seen (baseline *P.aeruginosa* infection and ≥4 exacerbations) created a subgroup demonstrating a significant response to therapy (50 PDPE’s in 34 erythromycin subjects vs 76 in 26 placebo subjects, mean 1.47 (0.98-1.97) vs 2.92 (2.06-3.79), difference 1.45 (0.48-2.42), p=0.005). Test of interaction showed a significant difference in effect compared with the remainder of subjects (treatment effect 1.45 vs 0.15, difference 1.30 (95% CI 0.11 to 2.49), p=0.032).

**FEV₁**

Given the possibility of variability in FEV₁, an additional post-hoc analysis was performed involving averaging all of the treatment period measurements of FEV₁ to determine a mean treatment period percent-predicted FEV₁ for each subject and the change from baseline then compared between the erythromycin and placebo arms. This method was a variation on the methods described in O’Donnell’s DNase study 7 (adapted because our pre-specified FEV₁ outcome variable was change in percent predicted FEV₁ rather than proportional/ percentage change in actual FEV₁ as described in that study). FEV₁ was assessed at every trial visit and hence this mean treatment period percent-predicted FEV₁ was taken from 7 visits (visits 2 to 8 inclusive). This analysis confirmed a significant effect of erythromycin on attenuating lung function decline over the treatment period (erythromycin mean 0.49% decline vs placebo 2.71% decline, treatment effect 2.08 (0.36 to 3.8)% p=0.012).

**Changes in sputum microbiology**

Tables E2 to E5 provide details of changes in sputum microbiology across the study. The emergence of new sputum pathogens after commencement of trial medication was similar in the erythromycin and placebo arms, both overall and for individual pathogens (see Table E3). However, eradication of sputum pathogens (negative
sputum culture in the visit 8 sputum sample, from subjects with pathogenic bacteria identified in pretreatment samples) occurred in significantly more erythromycin-treated subjects (see Table E4).

Changes in outcome measures in the 4-week washout period
All of the following analyses relate to comparisons of results obtained at the completion of the 4 week washout (visit 9) compared with the visit at the completion of the treatment period (visit 8).

There were no differences between placebo and erythromycin groups for the occurrence of PDPE’s in the 4 week washout period with a total of 27 events (erythromycin 14, placebo 13).

Washout from erythromycin therapy was associated with a significant increase in 24 hour sputum production when comparing visits 9 and 8 for within-group change (10.6 (9.9 to 14.9) g vs 8.3 (7.1 to 10.5) g, p=0.001 by Wilcoxon’s signed ranks test, see Figure S2), however this increase was not significantly different from the placebo arm change (erythromycin increase 2.2 (0.6 to 5.6) g vs placebo 0.9 (-0.1 to 2.2) g, median difference 0.9 (-1.5 to 3.2), p=0.47).

The change in post-bronchodilator percent–predicted FEV₁ during the washout period did not differ significantly between erythromycin and placebo (erythromycin arm change 0 (-1.86 to 0.98) vs -0.60 (-3.69 to 0.87) %, difference 0.56 (-1.33 to 2.52), p=0.53).

There was a significant deterioration in the LCQ score following cessation of erythromycin (15.25 (14.79 to 18.09) vs 16.55 (14.45 to 16.49), p=0.0088 by Wilcoxon’s signed ranks test) however this deterioration was not significantly different from the change with placebo (erythromycin -0.7 (-1.54 to -0.04) vs placebo -0.33 (-0.75 to 0.12), -0.34 to 1.15, p=0.27). A clinically significant deterioration in the LCQ (≥1.3 decline) occurred in 22 (41.5%) erythromycin vs 13 (24.5%) placebo subjects (OR 2.18, 95% CI 0.95 to 5.09, p=0.068 by Fisher’s exact test).

One month’s washout from erythromycin therapy resulted in a significant deterioration in SGRQ symptoms score (within group comparison: 57.0 (51.5 to 61.8) vs 49.1 (44.1 to 54.1), change 7.9 (2.5 to 13.2), p=0.005). This change trended to significance compared with the change in the placebo arm (7.9 (2.5 to 13.2) vs 1.7 (-3.5 to 7.0), difference 6.1 (-1.2 to 13.5), p=0.10).

SGRQ total score also deteriorated significantly on within-group analysis in the erythromycin arm (35.0 (30.9 to 38.6) vs 32.2 (28.6, 35.8), change 2.8 (0.3 to 5.3), p=0.03), but this change did not differ from the change in the placebo arm (2.8 (0.3 to 5.3) vs 1.5 (-1.1 to 4.1), difference 1.3 (-2.3 to 4.9), p=0.46).
eFigure 1. Study schema

Screen

Visit Number

Week

V0 V1 V2 V3 V4 V5 V6 V7 V8 V9

0 4 8 16 24 32 40 48 52

Washout (4 weeks)

Phone contact/ reviews monthly between visits
eFigure 2. Mean scores for the Leicester Cough Questionnaire across the study

(Lower scores indicate worse cough symptoms. N=58 placebo, 59 erythromycin. The difference in change between V1 and V8, comparing the two arms, did not reach statistical significance. Error bars represent 95% CI)
eTable 1. Summarised event rates for pulmonary exacerbations

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean  Median (IQR)</td>
<td>N  Mean  Median (IQR)</td>
</tr>
<tr>
<td>PDPE’s – total</td>
<td>58 1.97   2 (0, 3)</td>
<td>59 1.29   1 (0, 2)</td>
</tr>
<tr>
<td>Total Respiratory Events (PDPE's + non-PDPE's) – total</td>
<td>58 3.03   3 (2, 4)</td>
<td>59 1.88   1 (1, 3)</td>
</tr>
<tr>
<td>Total Respiratory Events (PDPE's + non-PDPE's) – per protocol</td>
<td>53 3.26   3 (2, 5)</td>
<td>52 1.83   1 (1, 3)</td>
</tr>
<tr>
<td>PDPE’s- Subgroup with P.aeruginosa at baseline</td>
<td>18 2.89   2 (2, 5)</td>
<td>23 1.57   1 (0, 3)</td>
</tr>
<tr>
<td>- Remainder (no P.aeruginosa at baseline)</td>
<td>40 1.55   1 (0, 2.5)</td>
<td>36 1.11   1 (0, 1.5))</td>
</tr>
<tr>
<td>PDPE’s - Subgroup with ≥5 exacerbations at baseline</td>
<td>20 3.05   2 (1.5, 5)</td>
<td>22 1.50   1 (0, 2)</td>
</tr>
<tr>
<td>- Remainder (2-4 exacerbations at baseline)</td>
<td>38 1.39   1 (0, 2)</td>
<td>37 1.16   1 (0, 2)</td>
</tr>
<tr>
<td>PDPE’s – subgroup with ≥5 exacerbations OR P.aeruginosa at baseline</td>
<td>26 2.92   2 (2, 5)</td>
<td>34 1.47   1 (0, 2)</td>
</tr>
<tr>
<td>- Remainder (neither P.aeruginosa nor ≥5 exacerbations)</td>
<td>32 1.19   1 (0, 2)</td>
<td>25 1.04   1 (0, 1)</td>
</tr>
</tbody>
</table>

(All results are on the ITT population, except where indicated otherwise)
eTable 2. Secondary outcome measures (all results presented are for the ITT population) with missing values assigned by multiple imputation analysis

<table>
<thead>
<tr>
<th>Measure</th>
<th>Placebo (n=58)</th>
<th>Erythromycin (n=59)</th>
<th>Treatment effect (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Post-bronchodilator FEV\textsubscript{1}, percent predicted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- mean (SD)</td>
<td>-3.8 (±6.6)</td>
<td>-1.3 (±4.5)</td>
<td>2.3 (0.3 to 4.4)*</td>
</tr>
<tr>
<td>- median (IQR)</td>
<td>-3.1 (0, -7.8)</td>
<td>-1.1 (1.6, -4.3)</td>
<td></td>
</tr>
<tr>
<td><strong>24 hour sputum weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– median (IQR)</td>
<td>-2.25 (-8.2, 2.5)</td>
<td>-5.1 (-15.5, -1.2)</td>
<td>-4.1 (-0.9 to -7.8)**</td>
</tr>
<tr>
<td><strong>SGRQ total</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- mean (SD)</td>
<td>-2.3 (±13.7)</td>
<td>-4.4 (±9.7)</td>
<td>-2.4 (1.8 to -6.7)</td>
</tr>
<tr>
<td>- median (IQR)</td>
<td>-3.2 (-8.8, 6.5)</td>
<td>-4.6 (-11.6, 4.1)</td>
<td></td>
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<tr>
<td><strong>SGRQ symptoms</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>- mean (SD)</td>
<td>-4.9 (±22.6)</td>
<td>-4.8 (±22.7)</td>
<td>-2.2 (4.8 to -9.2)</td>
</tr>
<tr>
<td>- median (IQR)</td>
<td>-0.4 (-19.2,11.0)</td>
<td>-7.3 (-18.4, 16.0)</td>
<td></td>
</tr>
<tr>
<td><strong>Leicester cough questionnaire</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>- mean (SD)</td>
<td>0.71 (±3.1)</td>
<td>1.21 (±3.1)</td>
<td>0.74 (-0.3 to 1.7)</td>
</tr>
<tr>
<td>- median (IQR)</td>
<td>0.51 (-37, 28)</td>
<td>1.5 (-70, 32)</td>
<td></td>
</tr>
<tr>
<td><strong>6MWT (m)</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>– mean (SD)</td>
<td>6.4 (±66.6)</td>
<td>0.1 (±57.4)</td>
<td>2.2 (-4.8 to 9.2)</td>
</tr>
<tr>
<td>- median (IQR)</td>
<td>2.5 (-37, 28)</td>
<td>2 (-20, 24)</td>
<td></td>
</tr>
<tr>
<td><strong>Percentage of macrolide resistant oropharyngeal streptococci</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>– median (IQR)</td>
<td>0 (-1.7, 2.0)</td>
<td>28.9 (1.5, 39.2)</td>
<td>25.6 (14.8 to 32.7)**</td>
</tr>
</tbody>
</table>

(Multiple imputation was performed by the Gaussian normal regression imputation method, using Stata/SE 12.0 for Windows; Plus-minus values are mean (±SD), else median (interquartile range). *p=0.026, ** p=0.016, *** p<0.0001, by ANCOVA excepting 24 hour sputum weight and macrolide-resistant streptococci both by Mann-Whitney U test. Treatment effect refers to the corrected mean difference or the unadjusted median difference between arms. FEV\textsubscript{1}-% - forced expiratory volume in the first second, as percentage of the predicted value; Leicester Cough Questionnaire - lower scores indicate worse cough symptoms, score range 3-21; SGRQ – St George’s Respiratory Questionnaire, lower scores indicate better quality of life; g – grams; 6MWT – six minute walk test.)
**eTable 3. Rates of culture of sputum pathogens at trial commencement and final visits**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Erythromycin</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Visit 1</td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>18 (31)</td>
<td>16 (28)</td>
</tr>
<tr>
<td><strong>H. influenzae</strong></td>
<td>14 (24)</td>
<td>12 (21)</td>
</tr>
<tr>
<td><strong>Normal respiratory flora only</strong></td>
<td>26 (45)</td>
<td>27 (47)</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>5</td>
<td>2</td>
</tr>
</tbody>
</table>

(Values given are number (percent). Baseline refers to either the screening or visit 1 sample. Final visit refers to visit 8, excepting subjects who discontinued early from the study and instead had sputum culture performed at discontinuation. For final visit samples, n=55 for placebo and 56 for erythromycin.)

**eTable 4. Individual subjects with new appearance of pathogens after study commencement**

<table>
<thead>
<tr>
<th></th>
<th>Placebo</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>New appearance of any pathogen at any time during study compared against baseline cultures</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>P. aeruginosa</strong></td>
<td>6</td>
<td>4</td>
</tr>
<tr>
<td><strong>H. influenzae</strong></td>
<td>4*</td>
<td>7*</td>
</tr>
<tr>
<td><strong>Other</strong></td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>12</td>
<td>13</td>
</tr>
</tbody>
</table>

(Baseline refers to either the screening or visit 1 sample. * Of these subjects, 1 placebo and 3 erythromycin subjects had instead cultured P. aeruginosa at baseline.)
eTable 5. Individual subjects with eradication of all sputum pathogens at the final visit

<table>
<thead>
<tr>
<th>Subjects in whom no pathogens cultured at final visit after being initially culture positive for any pathogen</th>
<th>OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>Erythromycin</td>
</tr>
<tr>
<td>Visit 1 culture positive</td>
<td>4 (7.3)</td>
</tr>
<tr>
<td>Baseline culture positive</td>
<td>6 (10.9)</td>
</tr>
</tbody>
</table>

(Values shown are number (%). Eradication describes no pathogens cultured in the final sputum sample after initially culturing any sputum pathogen in pre-treatment samples. Baseline refers to either the screening or visit 1 sample. Final visit refers to visit 8, excepting subjects who discontinued early from the study and instead had sputum culture performed at discontinuation. For final visit samples, n=55 for placebo and 56 for erythromycin. *p<0.05 for the comparison of erythromycin and placebo arms.)

eTable 6. Changes in *P. aeruginosa* and *H. influenzae* sputum cultures in individual subjects during the study

<table>
<thead>
<tr>
<th>Subjects in whom organism cultured at baseline but not at V8 ('eradication' of individual pathogens)</th>
<th>Placebo</th>
<th>Erythromycin</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. aeruginosa</em></td>
<td>3</td>
<td>7*</td>
</tr>
<tr>
<td><em>H. influenzae</em></td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

(Baseline refers to either the screening or visit 1 sample. There were no other individual organisms for which changes in culture results occurred in more than a single subject. * 1 erythromycin subject instead cultured *H. influenzae* at this final visit.)
References


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