A two year randomised placebo controlled trial of doxycycline for lymphangioleiomyomatosis

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Key finding: Doxycycline causes modest reductions in metalloproteinases but does not prevent FEV₁ decline in a placebo controlled trial in LAM

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Abstract

**Rationale** Lymphangioleiomyomatosis (LAM) is characterised by lung cysts and airflow obstruction. Matrix metalloproteinases (MMPs) have been implicated in lung destruction in LAM. We performed a randomised, double blind trial, comparing the MMP inhibitor doxycycline with placebo on the progression of LAM.

**Methods** Twenty three women with LAM were randomised to doxycycline 100mg daily for three months followed by 200mg daily for 21 months, or matched placebo. Lung function, exercise capacity, quality of life and MMP levels were measured.

**Main results** Twenty one patients completed 6 months treatment, 17 completed 1 year and 15 completed 2 years. Four withdrew due to pneumothorax and four for other reasons. Mean decline in FEV₁, the primary endpoint, did not differ between the groups being -90 (SD 154) ml/year in the placebo group and -123 (246) ml/year in the doxycycline group (difference -32.5, 95% C.I. -213 to 148, p=0.35). Doxycycline had no effect upon vital capacity, gas transfer, shuttle walk distance or quality of life. Urine MMP-9 measurements were lower with doxycycline treatment (p=0.03).

**Conclusions** Although with limited numbers we cannot completely exclude an effect of doxycycline, the lack of effect on any outcome makes it unlikely that doxycycline has a useful effect in LAM.
Introduction

Lymphangioleiomyomatosis (LAM), a rare disease of the lungs and lymphatics which occurs almost exclusively in women, can occur as a sporadic disease or in patients with tuberous sclerosis complex (TSC). Pulmonary symptoms generally dominate the clinical picture as lung cysts form, causing pneumothorax, airflow obstruction and progressive respiratory impairment.[1] The rate of disease progression varies considerably between patients, with the decline in FEV₁ usually being between 70 and 140 ml/yr.[2-4] Lymphatic obstruction can lead to chylous pleural effusions, ascites and abdomino-pelvic masses and around half of patients have renal angiomyolipomas, a benign mesenchymal tumour.[1]

In patients with LAM the lungs and lymphatics are infiltrated by LAM cells, a clonal cell population with bi-allelic inactivation of the TSC-2 gene leading to constitutive activation of mTOR.[5] Targeting mTOR with sirolimus in patients with LAM has been shown to reduce the decline in FEV₁ and angiomyolipoma volume.[4, 6, 7]

LAM cells produce proteolytic enzymes which may contribute to lung cyst formation.[8, 9] Matrix metalloproteinases (MMPs) can degrade extra-cellular matrix, and can affect cell growth, invasion, angiogenesis and inflammation.[10] MMP-2 and -9 are overexpressed in the serum of women with LAM [11, 12] and MMPs -1, -2, -9 and -14 are strongly expressed in the lungs of patients, particularly adjacent to cysts where disrupted collagen and elastic fibres are seen.[13, 14] Inhibition of MMP activity could therefore reduce lung destruction in LAM.

Doxycycline, a tetracycline antibiotic, inhibits the production and activity of several MMPs including MMP-1, -2 and -9, and it has reduced pathological tissue remodelling in models of vascular disease and tumour growth.[15, 16] Doxycycline is the only MMP inhibitor licensed for clinical use and Moses et. al. described a large improvement in spirometry and oxygenation in a patient with advanced LAM following treatment with doxycycline.[17] In an observational study of patients with mild LAM, doxycycline was
associated with a reduction in urine MMP-9 and a relatively slow decline in mean FEV₁ of 70 ml over 12 months.[18, 19] As a consequence of these reports, some women with LAM have been taking doxycycline off label. In order to determine if doxycycline could inhibit MMP activity and reduce lung destruction in LAM, we conducted a randomised study of doxycycline and placebo over two years, using rate of decline in FEV₁ (ΔFEV₁) as the primary outcome measure. Other physiological measures, quality of life, MMP activity, safety and tolerability were also recorded.
Methods

Study protocol

Patients were eligible for the study if they were over 18 years and had sporadic or TSC associated LAM, classified as ‘definite’ by European Respiratory Society criteria,[20] and an FEV1 below 80% predicted or evidence of a 20% deterioration in FEV1. Patients were excluded if they were post lung transplant or if they had used mTOR inhibitors or had had a pneumothorax, chylous effusion or bleeding angiomyolipoma within the previous three months. Hormone and bronchodilator treatment for LAM was allowed providing treatment had not changed in the three months prior to enrolment. The study was approved by the Trent Multicentre Research Ethics committee (NRES 07/H0403/165) the Medicines and Healthcare Regulatory Agency (MHRA 03057/0032/001-002) and registered with the EU Clinical Trials Registry (EUDRACT 2007-003745-32). All patients provided informed consent.

Patients had a medical history and physical examination and completed a St Georges Respiratory Questionnaire (SGRQ) at baseline. Pulmonary function tests including post bronchodilator spirometry, gas transfer, lung volumes and endurance shuttle walk test were measured according to ARTP/BTS standards in a single laboratory.[21] Blood was drawn for haematology, biochemistry, liver function, C reactive protein and blood and urine for analysis of MMPs and other biomarkers.

Patients were randomised to receive either doxycycline 100mg daily or matched placebo as a single tablet. After 3 months the dose was increased to two tablets of the active drug (200mg doxycycline) or placebo. Patients were assessed every three months over two years. At 12 and 24 months, patients had a full evaluation as at baseline (figure 1). A CT scan of the thorax and abdomen was carried out at 0 and 24 months in patients giving additional consent. The longest dimension of the largest renal angiomyolipoma was measured by a radiologist (MK) as described.[7] Patients were withdrawn from the
study if there was a fall in FEV$_1$ from study baseline of more than 300ml on two consecutive visits or other severe adverse event. Those experiencing pneumothorax were also withdrawn from the study, as spirometry can take many months to return to baseline following pneumothorax (unpublished data). The full protocol is available in the online supplement.

**MMP and VEGF-D measurements**

Serum total MMPs and vascular endothelial growth factor-D (VEGF-D) were measured using a Quantikine MMP-2 Immunoassay, Duoset Human MMP-9 Immunoassay and Human VEGF-D ELISA respectively (all from R&D Systems, Minneapolis, MN). Since MMPs are secreted as inactive zymogens which require proteolytic cleavage for activation, we measured pro and activated MMP-9 in serum and urine using gelatin zymography as described.[12, 22]

**Analyses**

The primary outcome was rate of decline in post bronchodilator FEV$_1$ over the course of the study [$\Delta$FEV$_1$] analysed on an intention to treat basis on all patients. $\Delta$FEV$_1$ was calculated by fitting a regression line to all post bronchodilator FEV$_1$ measurements for each patient with the slope of this line expressed in ml/yr. The effect of loss to follow up was examined by comparing $\Delta$FEV$_1$ between groups who only completed 6, 12, 18 and 24 months of treatment. For all endpoints Normality was assessed by Kolmogorov-Smirnov statistic and mean values for the two groups were compared by two sample t-test. Analyses were performed in Graphpad Prism version 5.00, (GraphPad Software, San Diego California USA). Serum and urine MMP values were compared as log transformed values over time between treatment groups using a linear, mixed model, with repeated measures from individuals as a random effect in Stata 11 (Timberlake Consultants, London, UK).
Results

**Patient recruitment and baseline characteristics**

Patients were recruited over a two year period starting in May 2009. After contacting 149 patients with LAM in the UK, 30 of those responding appeared to be suitable and were screened, of whom 23 were eligible. Twelve were assigned to take doxycycline and 11 to take matched placebo (figure 2). At recruitment the mean age of patients was 46 years and symptoms had been present for an average of 13.5 years. Eighteen (78%) patients had had a pneumothorax in the past and 13 (56%) had or had had an angiomyolipoma. A third of patents were post menopausal. One patient had tuberous sclerosis complex and the remainder sporadic LAM. Twenty one of the 23 patients had a serum VEGF-D level greater than the figure of 800 pg/ml considered diagnostic for LAM. [23] Patients had moderate to severe airflow obstruction with a mean FEV$_1$ of 1.69 l (58% predicted) and moderately impaired gas transfer of 4.38 kPa/min/ml (51% predicted). Baseline characteristics within the two groups were similar in terms of age, disease duration, clinical manifestations, menopausal status, quality of life and serum VEGF-D but mean FEV$_1$ and TL$_{CO}$ were slightly lower in the doxycycline group (tables 1 and E1).

**Effect of doxycycline on rate of decline of FEV$_1$**

Rate of decline in FEV$_1$ ($\Delta$FEV$_1$) analysed on an intention to treat basis was -90.3 (SD 154) ml/yr in the placebo group and -123 (246) in the doxycycline group; the difference, -32.5 ml/yr (95%CI -213 to 147.8), was not significant (p=0.35, figure 3). Patients not completing the study had a greater decline in FEV$_1$ (figure 4). Mean $\Delta$FEV$_1$ was -36.3 (SD 63) ml/year for all patients completing the study and -240 (302) for those stopping early for any reason (p=0.049): median values were -30 ml/yr and -162 ml/yr respectively. A sensitivity analysis showed there was no difference between doxycycline and placebo for any duration of treatment for the primary endpoint (table 2).
Secondary endpoints

Patients treated with doxycycline showed a 150ml rise in FVC after 12 months compared with placebo treatment, although no difference was present at 24 months. Otherwise, there were no differences between change in TLCO, shuttle walk distance or in quality of life scores between the two groups after 12 or 24 months of treatment (table 3). VEGF-D did not change significantly in either group over the course of the study (table 3). Twenty patients underwent CT scanning of the chest and abdomen at baseline and 13 had a further scan at the end of the study. No LAM related complications including chylous collections developed during the study in any patient. A follow up renal scan in six patients receiving placebo and six receiving doxycycline showed no change in mean angiomyolipoma size in either group (figure E1).

Adverse events

Adverse events were reported on all patients. Six patients were withdrawn because of adverse events. In four this was due to a pneumothorax, (one doxycycline, three placebo), in one patient to a fall in FEV1 of greater than 300ml (doxycycline) and one patient receiving doxycycline had an epileptic seizure and was found to have a meningioma (figure 2). Three patients in the placebo group and three in the doxycycline group had at least one respiratory infection requiring antibiotic treatment over the study period, with two patients in the doxycycline group having several respiratory infections (table 4). Although more adverse events were reported with doxycycline, only dyspepsia and photosensitivity were attributed to the drug. No significant disturbances in haematologic or biochemical values occurred in either group.

Effect of doxycycline on serum and urine MMPs

At baseline MMP-2 and MMP-9 were present in serum on gelatin zymography (figure 5), and mean total serum MMP-2 and MMP-9 levels, measured by ELISA, were 259 (SD 48) and 265 (173) ng/ml respectively. In urine, MMP-9 dimers, neutrophil gelatinase-
associated lipocalin (NGAL) bound MMP-9, pro-MMP-9 and active MMP-9 were found, but not MMP-2 (figure 5).

There was no significant difference in serum MMP-2 or -9 between groups over the 2 year period (figure E3). Urine total and active MMP-9 values varied markedly between subjects at baseline and within subjects during the study in patients receiving placebo. This variation did not relate to infections as assessed clinically or by CRP and neutrophil counts (figure 5 and E4). There was a significant reduction in total urinary MMP-9 ($p=0.03$) and a reduction of borderline significance in active MMP-9 ($p=0.07$) in the doxycycline group when compared with placebo values over the 2 year period (figure 5).
Discussion

We have conducted the first randomised, placebo controlled trial of doxycycline as a potential therapy for LAM. Doxycycline had no effect on the rate of decline of FEV$_1$ over two years, and no effect on FVC, TL$\text{CO}_2$, total lung capacity, shuttle-walk distance or quality of life scores after 24 months of treatment. Because LAM is rare, the number of patients studied was relatively small. Our findings cannot therefore completely exclude an effect of doxycycline, although the lack of a sustained effect on any outcome makes it very unlikely that doxycycline has a useful effect that we have missed.

Study subjects were drawn from a national database and a clinical referral service that has evaluated over half the patients known to have LAM in the UK. Patients were similar to those in other studies, being in their mid-forties, with a disease duration of thirteen years and with a similar prevalence of pneumothorax and angiomyolipoma.[24] Patients had moderate air-flow obstruction and impairment of gas transfer. Initial power calculations suggested we would require 20 patients per group to have 80% power to detect a 50% reduction in $\Delta$FEV$_1$. Performing studies in rare diseases is difficult as patients need to be from a large area to deliver adequate study power, and only one randomised study of LAM has been published to date. Frequent pneumothoraces also limit recruitment of patients with LAM. Our recruitment criteria were broad and we linked study visits with the patients’ medical care where possible to facilitate participation in the study. Performing the study in a single centre ensures that procedures are standardised but the need for patients to travel limited recruitment to some extent. A more significant issue was that mTOR inhibitor therapy became more widely available during the recruitment period and the enlarging evidence base for this therapy limited our ability to recruit patients with progressive disease to a placebo controlled study.[6, 25] The study was designed to guard against patients with rapidly declining lung function receiving placebo for two years by incorporating stopping criteria for patients with a greater than...
300ml fall in FEV₁ as a pre-determined secondary end point. Two patients left the study early to receive sirolimus, one after a greater than 300ml fall in FEV₁.

In the intention to treat analysis mean $\Delta$FEV₁ was -123 ml/year in the doxycycline group and -90 ml/year in the placebo group, values in keeping with previous reports. Patients who withdrew early had more rapid decline in lung function. The relatively low $\Delta$FEV₁ in patients completing the study, -35 ml/yr, reflects the fact that patients with more aggressive disease were already receiving sirolimus or were opting for it. In addition, one third of our study population were post-menopausal when the decline in FEV₁ is slower which may make it more difficult to show a treatment effect. [2, 3] The 95% CI for $\Delta$FEV₁ in the patients completing the study were tighter than in the intention to treat analysis, despite the smaller numbers, reflecting the fact that more measurements were made per patient over a longer period, and these patients had more stable disease. Nevertheless there was little difference in mean $\Delta$FEV₁ between the two groups (6.1 ml/yr (95% CI -67 to 79)). A limitation of the study was that, due to chance, patients randomised to doxycycline had slightly lower lung function than those receiving placebo. Although there is no definitive data on whether lung function varies with disease severity in LAM, it is possible that those with more severe disease may decline more rapidly although the converse is true in patients with alpha 1-anti-trypsin deficiency. [26]

Two reports have described the effect of doxycycline on lung function. In a single patient with severe disease, lung function and oxygenation improved after doxycycline treatment although this may have been due treatment of a co-existing infection. [17] In a series of 38 patients all treated with doxycycline for 12 months, Pimenta et. al. observed that some patients remained stable on treatment and these tended to be those with better lung function. A relatively benign clinical course in some patients with LAM is well documented, and without a control group the significance of these findings is difficult to assess. [27] MMP-2 and -9 are increased in LAM lung tissue,[14] in cells
derived from TSC knock-out animals[28] and in the serum and urine of patients with LAM,[11] making them attractive candidates for causing the accelerated extra-cellular matrix destruction and cyst formation observed.[13, 14, 29] All the MMP measurements, but particularly those from urine, showed considerable intra and inter-subject variation which was not obviously related to infection. Doxycycline treatment was associated with suppression of urinary MMP-9 but not serum MMP-9. Our failure to find a reduction in serum MMP-9 contrasts with the 5% reduction seen by Pimenta et. al.[19] and although this may reflect our smaller numbers and intra-subject variability, there must be doubt as to whether a change of 5% would result in clinical benefit. The lack of efficacy of doxycycline in preventing decline in lung function raises questions about the role of MMPs in lung destruction in LAM. However, the metalloproteinase system is complex with activating proteases and inhibitors interacting to regulate overall MMP activity both spatially and temporally. Further studies are required to determine whether MMPs are central to lung destruction in LAM and if more potent or selective targeting of individual proteases or their substrates could reduce lung destruction.

In summary, we found that treatment with doxycycline for two years had no effect on the decline in lung function in patients with LAM. It is common for patients with a rare disease to take off-label therapies on the basis of a biologically plausible mechanism of action but our findings provide no support to justify using doxycycline to treat LAM. Specific targeting of lung destruction in LAM and other chronic lung diseases needs better understanding of the pathologic mechanisms involved.
**Acknowledgements**

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**Funding**

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Figure 1. Outline of study protocol. Visit includes history, physical examination, post bronchodilator spirometry and safety blood tests. QoL: Quality of life assessment.
figure 1
Figure 2. Recruitment and retention of participants.
Figure 3. Serial post bronchodilator FEV$_1$ for all study participants.
Figure 4. ΔFEV₁ for individual participants compared with time to study withdrawal.

Patients with more rapid falls in FEV₁ were more likely to withdraw from the study.
Duration of study (months)

Δ FEV1 (mL/yr)

○ placebo
● doxycycline

-884

figure 4
Figure 5. (a) Gelatin zymograms of serum and urine from representative patients treated with doxycycline or placebo to detect MMP-2 and -9. MMP species are visible as white areas of degraded gelatin. rhMMP: recombinant human MMP protein standard. NGAL: neutrophil gelatinase associated lipocalin. (b) Mean (+SE) urinary total and active MMP-9 throughout the two years of the study. P values are for overall differences between treatment groups.
Figure 5
## Tables

Table 1.

Baseline characteristics of study subjects

<table>
<thead>
<tr>
<th></th>
<th>All patients (S.D.)</th>
<th>Doxycycline (S.D.)</th>
<th>Placebo (S.D.)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>n</strong></td>
<td>23</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td><strong>Patient characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age - yr</td>
<td>46.5 (9.0)</td>
<td>47.0 (9.3)</td>
<td>45.7 (8.9)</td>
</tr>
<tr>
<td>Duration of disease - yr</td>
<td>13.5 (9.1)</td>
<td>14.5 (9.0)</td>
<td>12.5 (9.4)</td>
</tr>
<tr>
<td>Post menopause</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Pneumothorax†</td>
<td>18</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Angiomyolipoma‡</td>
<td>14</td>
<td>6</td>
<td>8</td>
</tr>
<tr>
<td>TSC-LAM</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Supplemental oxygen use</td>
<td>5</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td><strong>Lung function - % predicted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁</td>
<td>58 (23)</td>
<td>52 (25)</td>
<td>64 (19)</td>
</tr>
<tr>
<td>FVC</td>
<td>95 (22)</td>
<td>97 (26)</td>
<td>92 (18)</td>
</tr>
<tr>
<td>TL_{CO}</td>
<td>51 (21)</td>
<td>45 (21)</td>
<td>57 (20)</td>
</tr>
<tr>
<td>TLC</td>
<td>94 (20)</td>
<td>99 (23)</td>
<td>89 (15)</td>
</tr>
<tr>
<td><strong>Other parameters</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shuttle walk dist. - m</td>
<td>573 (286)</td>
<td>560 (284)</td>
<td>586 (301)</td>
</tr>
<tr>
<td>SGRQ - total score</td>
<td>35 (4)</td>
<td>36 (6)</td>
<td>33 (6)</td>
</tr>
<tr>
<td>VEGF-D - pg/ml</td>
<td>2540 (1377)</td>
<td>2347 (1399)</td>
<td>2751 (1387)</td>
</tr>
</tbody>
</table>

† Number of patients having had at least one pneumothorax. ‡ Number of patients with one or more angiomyolipoma at any time.
Table 2.

Sensitivity analysis comparing $\Delta$FEV$_1$ in each group for varying durations of treatment.

<table>
<thead>
<tr>
<th>Duration of treatment</th>
<th>n</th>
<th>mean $\Delta$FEV$_1$ ml/yr (SD)</th>
<th>mean difference 95% C.I.</th>
<th>p</th>
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<tr>
<td>Any (ITT analysis)</td>
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<td></td>
<td></td>
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<tr>
<td>Doxycycline</td>
<td>12</td>
<td>-122.8 (246)</td>
<td>-32.5 -213 to 147.8</td>
<td>0.35</td>
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<td>Placebo</td>
<td>11</td>
<td>-90.3 (154)</td>
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<tr>
<td>&gt;6 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Doxycycline</td>
<td>12</td>
<td>-122.8 (246)</td>
<td>-61.3 -247 to 124</td>
<td>0.25</td>
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<tr>
<td>Placebo</td>
<td>9</td>
<td>-61.5 (112)</td>
<td></td>
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<tr>
<td>&gt;12 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>9</td>
<td>-34.4 (81)</td>
<td>-7.7 -76 to 60</td>
<td>0.41</td>
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<td>Placebo</td>
<td>8</td>
<td>-26.7 (42)</td>
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<tr>
<td>&gt;18 months</td>
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<td></td>
<td></td>
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<tr>
<td>Doxycycline</td>
<td>8</td>
<td>-33.5 (86)</td>
<td>6.1 -67 to 79</td>
<td>0.43</td>
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<tr>
<td>Placebo</td>
<td>7</td>
<td>-39.6 (24)</td>
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<td>24 months</td>
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<tr>
<td>Doxycycline</td>
<td>8</td>
<td>-33.5 (86)</td>
<td>6.1 -67 to 79</td>
<td>0.43</td>
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<tr>
<td>Placebo</td>
<td>7</td>
<td>-39.6 (24)</td>
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</table>

ITT: intention to treat
Table 3. Mean values and change at 12 and 24 months for secondary outcomes.

<table>
<thead>
<tr>
<th></th>
<th>12 months</th>
<th></th>
<th>24 months</th>
<th></th>
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<td>Doxycycline</td>
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<td>Placebo</td>
<td>Mean diff. for change</td>
<td>Dox vs. Pl</td>
<td>95% C.I.</td>
<td>p</td>
<td>Doxycycline</td>
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<td>Placebo</td>
<td>Mean diff. for change</td>
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<td>mean (SD)</td>
<td>change (SD)</td>
<td>mean (SD)</td>
<td>change (SD)</td>
<td>mean (SD)</td>
<td>change (SD)</td>
<td>mean (SD)</td>
<td>change (SD)</td>
<td>Mean diff. for change</td>
<td>Dox vs. Pl</td>
<td>95% C.I.</td>
</tr>
<tr>
<td><strong>Number completing</strong></td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td>7</td>
<td>3.09 (0.78)</td>
<td>0.19 (0.10)</td>
<td>3.04 (0.68)</td>
</tr>
<tr>
<td>FVC (l)</td>
<td>3.09 (0.78)</td>
<td>0.19 (0.10)</td>
<td>3.04 (0.68)</td>
<td>0.05 (0.11)</td>
<td>0.15</td>
<td>0.008</td>
<td>3.05 (0.83)</td>
<td>0.05 (0.18)</td>
<td>3.09 (0.74)</td>
<td>-0.004</td>
<td>-0.05</td>
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<td>TLco (mmol/kPa/min)</td>
<td>3.83 (1.98)</td>
<td>-0.02 (0.27)</td>
<td>4.91 (1.87)</td>
<td>0.16 (0.33)</td>
<td>0.18</td>
<td>0.11</td>
<td>4.23 (2.00)</td>
<td>-0.04 (0.56)</td>
<td>5.31 (1.47)</td>
<td>0.08</td>
<td>0.11</td>
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<tr>
<td>Shuttle walk distance (m)</td>
<td>617 (338)</td>
<td>33 (145)</td>
<td>554 (369)</td>
<td>-13 (139)</td>
<td>-46</td>
<td>0.25</td>
<td>632 (340)</td>
<td>4 (174)</td>
<td>630 (232)</td>
<td>-1</td>
<td>-5.18</td>
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<td>SGRQ symptoms</td>
<td>42.2 (30)</td>
<td>-0.8 (16.9)</td>
<td>32.5 (21.7)</td>
<td>0.48 (8.6)</td>
<td>-1.24</td>
<td>0.43</td>
<td>37.4 (20.4)</td>
<td>5.0 (14.7)</td>
<td>35.4 (25.4)</td>
<td>-7.1</td>
<td>12.1</td>
</tr>
<tr>
<td>activity</td>
<td>50.8 (32.3)</td>
<td>-1.7 (7.5)</td>
<td>51.2 (26.1)</td>
<td>4.7 (9.7)</td>
<td>-5.8</td>
<td>0.08</td>
<td>48.3 (25.9)</td>
<td>0.78 (12.2)</td>
<td>51.1 (22.5)</td>
<td>7.1</td>
<td>-6.3</td>
</tr>
<tr>
<td>impact</td>
<td>30.2 (24.9)</td>
<td>-3.7 (11.8)</td>
<td>25.1 (18.9)</td>
<td>-2.8 (7.3)</td>
<td>-10.6 to 9.1</td>
<td>0.44</td>
<td>26.5 (18.0)</td>
<td>2.1 (4.9)</td>
<td>22.7 (11.8)</td>
<td>0.7</td>
<td>1.2</td>
</tr>
<tr>
<td>total score</td>
<td>40.2 (27.6)</td>
<td>-3.1 (9.8)</td>
<td>32.0 (20.7)</td>
<td>-0.05 (4.9)</td>
<td>-11.4 to 5.3</td>
<td>0.22</td>
<td>32.2 (21.4)</td>
<td>1.7 (6.9)</td>
<td>31.4 (16.6)</td>
<td>1.3</td>
<td>-4.5 to 6.9</td>
</tr>
<tr>
<td>VEGF-D (pg/ml)</td>
<td>2135 (1256)</td>
<td>-107 (246)</td>
<td>2751 (1387)</td>
<td>-69 (981)</td>
<td>38</td>
<td>0.91</td>
<td>2229 (1083)</td>
<td>-412 (562)</td>
<td>2660 (1427)</td>
<td>-290</td>
<td>122</td>
</tr>
</tbody>
</table>

SGRQ: St Georges Respiratory Questionnaire. VEGF-D: vascular endothelial growth factor-D. Dox: doxycycline. Pl: placebo. P values are for the difference between the changes for doxycycline and placebo at the two time points.
Adverse events during study

Table 4.

<table>
<thead>
<tr>
<th>Category</th>
<th>All patients</th>
<th>Doxycycline grade</th>
<th>Placebo grade</th>
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</thead>
<tbody>
<tr>
<td></td>
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<td>1-2</td>
<td>3-4</td>
</tr>
<tr>
<td><strong>Auditory</strong></td>
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<tr>
<td>Tinnitus*</td>
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<tr>
<td><strong>Dermatology</strong></td>
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<tr>
<td>Dry skin</td>
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<tr>
<td>Flushing+</td>
<td>3</td>
<td>2</td>
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</tr>
<tr>
<td>Photosensitive rash</td>
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<td>Bruising</td>
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<tr>
<td><strong>GI</strong></td>
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<tr>
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<tr>
<td>Dyspepsia</td>
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<tr>
<td><strong>Infection</strong></td>
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<tr>
<td><strong>Lymphatics</strong></td>
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<tr>
<td>Chyle leak (chyloptysis)</td>
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<tr>
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<tr>
<td><strong>Pain</strong></td>
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<tr>
<td>Musculoskeletal</td>
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<tr>
<td><strong>Respiratory</strong></td>
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<tr>
<td>Dyspnoea</td>
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<td>1</td>
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<tr>
<td>FEV$_1$ (fall)</td>
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<tr>
<td>Pneumothorax</td>
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<td>Bronchospasm</td>
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<td>1</td>
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<tr>
<td><strong>Malignancy</strong></td>
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</tr>
<tr>
<td>Meningioma</td>
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<td>1</td>
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</tr>
<tr>
<td><strong>Total</strong></td>
<td>45</td>
<td>29</td>
<td>2</td>
</tr>
</tbody>
</table>

Adverse events categorised by Common Terminology Criteria for Adverse Events v3.0. No grade 5 events were observed. *Patient had tinnitus prior to study, symptoms persisted on stopping drug. $In a patient with meningioma. +All patients were approaching the menopause.
References


