A two year randomised placebo controlled trial of doxycycline for lymphangioleiomyomatosis

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On line data supplement

STUDY PROTOCOL

Title A randomised, double blind, placebo controlled trial of doxycycline in lymphangioleiomyomatosis.

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Summary

We will perform a 2 year double blind, placebo controlled trial of doxycycline in 40 patients with LAM. The main endpoints will be change in FEV_1, other measures of efficacy, safety and dose required to suppress MMP activity. After clinical evaluation, lung function, shuttle walk, QoL assessment, blood tests, serum and urine MMPs (termed full assessment) plus baseline chest X-ray patients will be randomised to doxycycline 50 mg od or placebo. Patients will be assessed at 2 weeks for a safety screen, every 3 months for clinical evaluation and spirometry and at 12 and 24 months for full assessment. At 1, 2 and 3 months urine zymography will be performed to see if MMPs are present in urine at the prescribed dose. Doxycycline will be increased to 50mg bd then 100 mg bd at monthly intervals up to three months after urine zymography has been performed. To avoid withholding treatment from those who decline rapidly, patients who, on two occasions, have either a fall from baseline FEV_1 of 300 ml or fall in resting SaO_2 of 3% will be assessed by an independent expert (AET). Patients receiving placebo will be given the option of doxycycline according to protocol. Those receiving doxycycline the option of continuing in the study or withdrawal. These patients and those withdrawn due to recurrent pneumothorax, increase in chylous effusion or bleeding angiomyolipoma will be included in a composite safety endpoint and analysed on an intention to treat basis. Power calculations based on retrospective cohorts[1] show that 20 patients per group will give 80% power to detect a 70 ml/year difference in FEV_1 based an assumed SD for a fall in FEV_1 of 75 ml/year. The mean slope of regression lines for FEV_1 and FVC, plus change DLCO, shuttle walk distance and QoL in the doxycycline and placebo groups will be compared by parametric or non-parametric analysis dependent on data, time to composite safety endpoint by Caplan-Meier analysis, complications and adverse events by Chi Square test.

Introduction

Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a disease of the lungs and lymphatics, which can occur sporadically or in association with tuberous sclerosis complex (TSC). The disease is rare, occurring in 1-2 / million of the population but in up to 40% of women with TSC LAM almost exclusively affects women, generally developing before the menopause. The disease is characterised by progressive pulmonary cystic change, recurrent pneumothorax, chylous pleural collections and, in most cases, progressive respiratory failure. Abdominal manifestations caused by obstruction and dilation of the axial lymphatics include lymphadenopathy, cystic lymphatic masses (lymphangioleiomyomas), chylous ascites and angiomyolipoma (a benign tumour). Survival in LAM is, 70 90% at 10 yrs, although this is highly variable since long-term survivors have been described. Diagnosis is made by a combination of clinical features and computed tomography scanning or, in cases of doubt, lung biopsy. In patients with rapidly progressive disease, hormone treatment (predominantly progesterone) has been used, although no firm evidence supports its use. Otherwise, treatment is aimed at complications including pneumothorax, chylous collections and extra-pulmonary manifestations. The only treatment for severe LAM is currently lung transplantation[2].

Recently identification of abnormalities in the tuberous sclerosis complex (TSC) genes in sporadic and TSC associated LAM have identified dysregulation of the mTOR pathway in LAM [3,4] and have lead to clinical trials of mTOR inhibitors such as rapamycin in LAM and TSC. At the time of writing these have not been reported but appear promising.

Background and preliminary data

Cystic lung destruction is the hallmark of pulmonary LAM and generally results in respiratory failure over a variable period of time[2]. Over-activity of proteases including elastase, trypsin and the matrix metalloproteinases (MMPs) is responsible for parenchymal destruction in emphysema and other lung diseases. The MMPs are a family of zinc dependent proteolytic enzymes with proteolytic activities against extra-cellular matrix proteins. The MMPs are overexpressed in inflammatory and neoplastic diseases where in addition to processing extra-cellular matrix they also have roles in metastasis, angiogenesis, growth factor activation and inactivation[5]. MMPs -1, -2, and -9 are involved in the sequential digestion of collagen and gelatin and are strongly expressed in the walls of cysts where it is thought they contribute to parenchymal destruction in LAM[6]. Further, MMPs can be detected by gelatin zymography in the urine of patients with LAM but not controls. Doxycycline is a tetracycline antibiotic in common clinical use. In addition to its antimicrobial action it inhibits the synthesis and activity of several MMPs and inhibits proliferation of a range of cell types including arterial smooth muscle, cancer cells and cancer model systems[7-9]. In preliminary experiments we have demonstrated that primary LAM derived and angiomyolipoma cells produce MMP-2 and -7 and that MMP expression and proliferation in these cells in inhibited by doxycycline.

Preliminary clinical data of doxycycline in LAM

In a preliminary open label study of doxycycline (50 - 100 mg qds) in 10 patients with LAM, doxycycline improved 6 minute walk distance) and Borg dyspnoea score (Glassberg et al. Data presented at the LAM Foundation International Research Conference, Cincinnati Ohio 2006). In a single case report, Moses et al. observed an improvement in FEV_1 and oxygenation during exercise in a patient with LAM treated with 100mg doxycycline daily [10]. In both of these cases MMP-2 and -9 were initially present in the patient's urine and was undetectable after treatment with doxycycline.

Specific issues in orphan disease clinical trials and rationale for trial design

Studying orphan diseases presents specific challenges, specifically the limited number of patients available, wide geographic distribution and low priority for funding due to the perceived poor economic benefit. Patients are well informed about potential developments due to patient groups and internet based information[11] and may obtain potential treatments 'off label' making definitive research studies impossible. As LAM is rare, cohorts drawn from a wide area are required for studies to achieve adequate power. We have 15 years experience of LAM research and from our UK LAM database estimate there are approximately 120 patients in the UK. We are currently performing an open label study of Sirolimus in LAM and tuberous sclerosis (TESSTAL, a Study of **T**he **E**fficacy and **S**afety of **S**irolimus (Rapamycin) **T**herapy for Renal Angiomyolipomas in Patients with **T**uberous Sclerosis Complex **A**nd Sporadic **L**ymhangioleiomyomatosis). This included six patients with LAM whom we found to be well motivated and will travel

long distances for study visits (including Cornwall, Kent and Perth). Despite the known adverse effects of rapamycin six of eight eligible (i.e. with LAM and angiomyolipoma) patients invited took part in the study. The current study protocol has been designed as a simple protocol which is both inclusive of most patients with LAM and has a follow up which is similar to routine clinical care which we hope will facilitate recruitment. In addition the protocol is less demanding than that of the TESSTAL study. As most patients are known to us via our database or clinical contacts we expect recruitment to be complete within six months.

Designing a definitive study is difficult without having an estimate of the likely size of effect, if this is large, as suggested by the one case report[10] a small number of patients are needed. If it is small as seems more likely *a priori*, a larger number of patients are needed requiring European collaboration and considerably greater funding. We therefore designed a pragmatic pilot study using a single geographic population with a simple, inexpensive protocol which will serve several functions, specifically to: (1) determine the optimum dose of doxycycline needed to suppress MMP production. (2) define the safety profile of doxycycline in LAM. (3) provide evidence of efficacy and size of effect. (4) provide data to help optimise the design and logistics of future trials.

2 Study aims/objectives

Hypothesis

Doxycycline will prevent matrix metalloproteinase dependent tissue destruction in lymphangioleiomyomatosis (LAM) thus preserving lung function, exercise capacity and quality of life.
We will perform a randomised placebo controlled trial of doxycycline on rate of decline of FEV_1 over two years against matched placebo. This study will:

(1) determine the optimum dose of doxycycline needed to suppress MMP production.
(2) define the safety profile of doxycycline in LAM.
(3) provide evidence for efficacy and size of effect.
(4) provide data to help optimise the design and logistics of future trials.

With the data obtained we will be in a strong position to apply for European funding for a European wide trial should this still be required.

3 Investigational plan

Patient population and recruitment

Forty patients with either sporadic LAM or TSC-LAM will be recruited from the UK LAM database, physician referrals, LAM Action (a patients group for women with LAM) and the Tuberous Sclerosis Association. Patients will be contacted by mail by the principal

investigator. Potential participants will receive a preliminary information sheet and response sheet for return by prepaid post. Those that express a potential interest in participating will be offered a face to face interview with one of the study doctors to assess eligibility, answer questions, obtain details of all physicians involved in their care and obtain written consent prior to enrolment. We anticipate recruitment will be complete within 12 months of starting the study.

Inclusion Criteria

- Sporadic LAM diagnosed either by cystic lung disease on HRCT classical of LAM plus angiomyolipoma or chylous effusion or cystic lung disease on HRCT and tissue biopsy showing LAM or angiomyolipoma
- TSC-LAM diagnosed by cystic lung disease on HRCT and tuberous sclerosis diagnosed by TSC consensus criteria[12].
- Patients with either an FEV_1 below 80% predicted or evidence of a 20% deterioration in FEV_1.
- Hormone and bronchodilator treatment for LAM* is allowed providing treatment has not changed in the three months prior to enrolment.
  * progesterone, GnRh agonists and bronchodilators.

Exclusion criteria

- Inability to give informed consent.
- Mental retardation.
- Age less than 18 years.
- Pneumothorax, chylous effusion, bleeding angiomyolipoma or change in hormone treatment within 3 months.
- Previous organ transplantation.
- Severe or uncontrolled epilepsy.
- Use of any oral contraceptive pill.
- Pregnancy or breast feeding. Pre-menopausal patients must be willing to use appropriate birth control measures to avoid pregnancy while enrolled in the study.
- Major systemic diseases (malignancy, myocardial infarction or unstable angina, type1 diabetes, severe hypertension, liver cirrhosis).
- Use of drugs known to interact with doxycycline, including anticoagulation with warfarin.
- Anticoagulation with warfarin.
- Hypersensitivity to tetracyclines.
- Treatment with mTOR inhibitor within the previous 3 months (sirolimus, everolimus).
- Use of doxycycline or other experimental drug within the previous three months.

<u>Sample size</u>

Power calculations have been based on several retrospective cohorts. In our UK national study the rate of decline in FEV_1 for all patients was 118 ml/yr (SD=142, n=43)[1] and 106 ml/yr (SD=143, n=57) in a similar French cohort[13]. In a larger study of less severely affected patients from the NIH the overall yearly rate of decline in FEV_1 was 75 ml/yr (SD=149, n=275)[14]. All of these studies have used data from retrospective series performed at different sites at different times. Using prospective standardised measurements performed by the same operators we expect the variation in change in FEV_1 to be smaller than observed in these cohorts. Using 20 patients per group there will 80% power to detect a difference of 70 ml/year difference in FEV_1 based on the assumption that the SD of the fall in FEV_1 in the trial is 75 ml/year.

Treatments/Interventions

Initial Assessment.
After giving informed consent, patients will have a baseline evaluation at a screening visit. This will include a history and physical examination by one of the study doctors, full lung function, shuttle walk (with SaO_2 monitoring), quality of life, FBC, U&E, LFT, glucose, calcium, serum MMP-2, -7, -9, urine zymography, serum for biomarkers and circulating LAM cell isolation (termed full assessment). In addition a baseline chest X-ray will be performed. All patients will have a pregnancy test.

Randomisation and initiation of doxycycline.
Patients will be randomised to either doxycycline 50 mg od or matched placebo. Randomisation will be performed by the University of Nottingham Clinical Trial Support Unit (CTSU).

Two week safety screen.
After two weeks of doxycycline treatment patients will have a safety screen comprising FBC, U&E, LFT, glucose, and amylase. A study phone call will be made to detect side effects and reinforce safety advice. Blood tests will be performed locally and sent to the centre in Nottingham.

Dose escalation of doxycycline and urine zymography.
In order to determine the dose of doxycycline that suppresses MMP production doxycycline will be increased and urine MMPs measured. After one month doxycycline will be increased from 50mg od to 50mg bd, after two months to 50 mg tds and at three months to 100 mg bd. Patients will stay on this dose for the remainder of the study. Blinding will be maintained during dose adjustment by prescribing all patients four study tablets being a combination of 50mg doxycycline and matched placebo tablets, dose adjustment will be performed by an independent person in a double blind manner. At one, two and three months urine will be sent by post to the study centre and zymography will be performed by a person blind to the clinical details of the patient. The group has a longstanding interest in the biology of MMPs in respiratory disease and expertise in MMP analysis[15-17]. In a preliminary study we have shown that urine MMPs are stable for greater than 30 hours at room temperature and can therefore be analysed at a site distant from collection (figure 1).

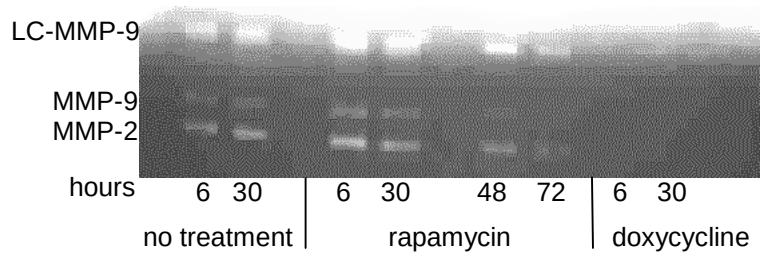


Figure 1. Urinary MMPs in LAM. Urine from three patients with LAM taking no treatment, rapamycin or doxycycline analysed by gelatin zymography after delays of 6-72 hours. MMPs are detectable in the urine of patients with no significant reduction up to 30 hours. The patient taking doxycycline has almost undetectable MMPs. LC-MMP-9: lipocalin associated MMP-9.

Routine study assessments.
Patients will be seen at the study centre every 3 months for clinical evaluation and spirometry (post bronchodilator). For safety purposes patients will have urinalysis, FBC, U&E, LFT, glucose, amylase and a pregnancy test. Patients with TSC will have a their epilepsy reviewed. At 12 and 24 months patients will undergo full assessment as described under initial assessment.

Monthly phone contact.
During months when no study visit is scheduled a telephone call will be made to participants to check compliance, reinforce safety advice and detect adverse events.

Combined safety endpoint.
To avoid withholding treatment from those who decline rapidly in the placebo group, patients with either a fall from baseline FEV_1 of 300 ml or a fall in resting SaO_2 of 3% (on two occasions) will be unblinded and offered doxycycline according to the protocol. Those deteriorating in the doxycycline group will be withdrawn from the study at the discretion of the doctor. In addition, patients with either pneumothorax, increase in chylous effusion or bleeding angiomyolipoma will be withdrawn. Patients fulfilling these criteria for withdrawal, together termed the 'composite safety endpoint', will be compared between treatment arms.

Endpoints

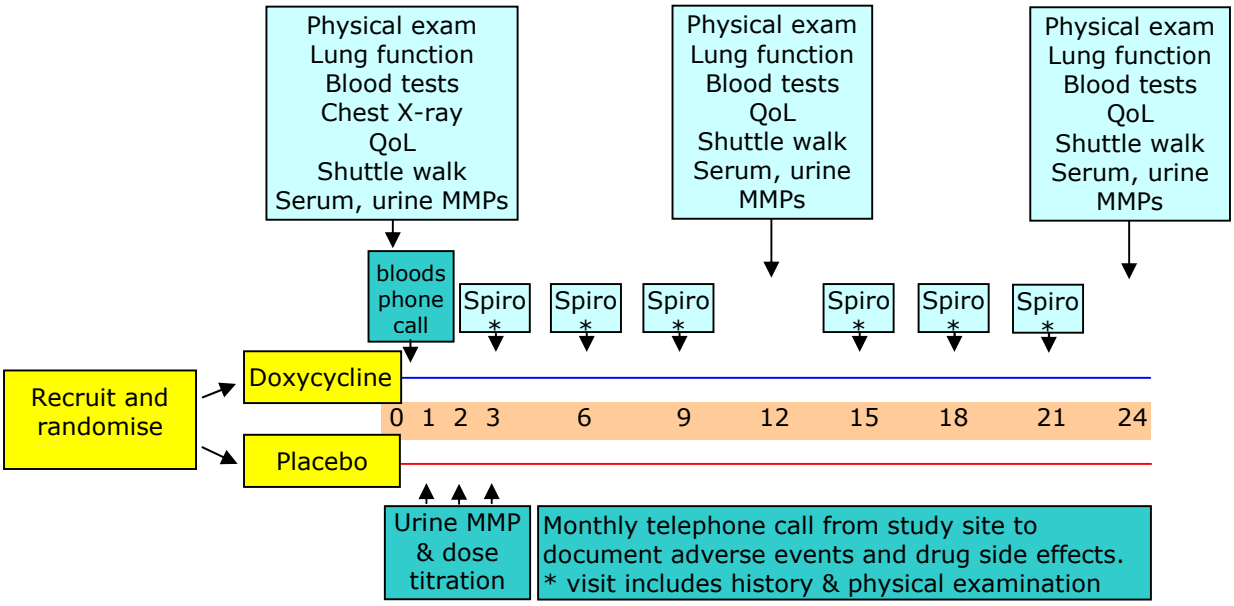
Primary endpoint.
Mean rate of change of FEV_1 over 24 months on doxycycline compared with placebo.

Secondary endpoints

Comparison between the two groups for:

- Rate of change of FVC over 24 months
- Change in DLCO at 12 and 24 months
- Change in shuttle walk distance at 12 and 24 months
- Change in quality of life at 12 and 24 months
- Time to composite safety endpoint
- Number of complications - pneumothorax, pleural effusions, angiomyolipoma bleeding
- Number of respiratory infections
- Adverse effects

Flow chart


Physical exam
Lung function
Blood tests
Chest X-ray
QoL
Shuttle walk
Serum, urine MMPs
Physical exam
Lung function
Blood tests
QoL
Shuttle walk
Serum, urine MMPs
Physical exam
Lung function
Blood tests
QoL
Shuttle walk
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Recruit and
randomise
Doxycycline
Placebo
0 1 2 3 6 9 12 15 18 21 24
Urine MMP
& dose
titration
Monthly telephone call from study site to
document adverse events and drug side effects.
* visit includes history & physical examination

Table2: Data capture summary

Visit (months)	Screening	2 wk	1	2	3	6	9	12	15	18	21	24
History and exam	✔				✔	✔	✔	✔	✔	✔	✔	✔
Blood tests (1)	✔	✔			✔	✔	✔	✔	✔	✔	✔	✔
Urine zymography	✔		✔	✔	✔			✔				✔
Pregnancy test	✔				✔	✔	✔	✔	✔	✔	✔	✔
Serum MMPs	✔							✔				✔
Quality of life	✔							✔				✔
Urinalysis	✔				✔	✔	✔	✔	✔	✔	✔	✔
Chest X-ray	✔											
Spirometry	✔				✔	✔	✔	✔	✔	✔	✔	✔
Full lung function (2)	✔							✔				✔
Review of epilepsy (3)	✔	✔			✔	✔	✔	✔	✔	✔	✔	✔

A study phone call will take place at 2 weeks, 4, 5, 7, 8, 10, 11,13, 14, 16, 17, 19, 20, 22, and 23 months to ensure no problems have developed.

1. renal profile, liver profile, bone profile, glucose, thyroid function, amylase, full blood count and differential. 2. Spirometry, lung volumes, gas transfer and shuttle walk test with oxygen saturation. 3 only in patients with TSC.

Study procedures

Medical history and examination.

At baseline and subsequent visits patients will undergo a full medical history concerning LAM, TSC and their complications, general medical history, drug treatment and sensitivities. At follow up visits patients will be specifically asked about compliance, any changes in medication and adverse events since the previous visit.

Blood tests.

At baseline, blood will be taken for FBC, U&E, LFT, glucose, calcium analysed in Chemical Pathology and Haematology Departments of the Queens Medical Centre, Nottingham using standard procedures.

At the same time serum will be taken for biomarker analysis, serum MMP-2, -9 and -7. These samples will be frozen and analysed by the study team at a later point using specific MMP ELISA (R&D systems) according to the manufacturer's instructions. Blood for biomarkers will be saved for later analysis.

Lung function tests. Spirometry, lung volumes, gas transfer and shuttle walk test will be performed by according to British Thoracic Society guidelines in the lung function unit Queens Medical Centre, Nottingham.

Spirometry. Spirometry alone will be performed according to BTS guidelines using a Vitalograph wedge bellows spirometer by a trained member of the study team.

Serum and Urine zymography. Zymography will be performed by a person independent of the study team using standard procedures. Briefly MMPs will be identified by gelatin zymography of serum and urine. Ten microlitres of sample (diluted

1:2 in 2x SDS sample buffer) will be run under non-denaturing conditions into precast Novex polyacrylamide zymogram gels (Invitrogen, Paisley, U.K.), supplemented with 0.1% gelatin with electrophoresis performed at a constant voltage of 125V for 90 min. Gels will be washed in re-naturing buffer and incubated overnight in developing buffer according to manufacturers guidelines, stained with 0.5% coomassie blue R-250 (in 50% water, 40% methanol; 10% acetic acid) and destained (in 50% water, 40% methanol; 10% acetic acid). Areas of gelatinolytic activity appearing as transparent bands will be analysed by densitometry and compared with a standard recombinant protein.

LAM Cell isolation. Heparinised blood is layered over histopaque (at 1:1 ratio) and centrifuged at 1500rpm for 30minutes at 22°C. The resulting buffy layer containing mononuclear cells is decanted into a 50ml tube and filled with HBSS. The cells are spun for 10mins at 1500rpm, the supernatant removed and the pellet resuspended in HBSS. After two further spins the resulting cells are cultured in fibronectin coated flasks. After 7-14 days resultant adherent cells are characterised by immunohistochemistry[18].

Quality of Life assessment. Will be assessed by the St Georges Respiratory Questionnaire and Chronic Respiratory Disease Questionnaire administered by the study team.

Pregnancy testing. Will be performed by urine βHCG analysis.

Storage of samples. All study samples will be stored in locked freezers in the Division of Therapeutics and Molecular Medicine, University of nottingham. All samples will be stored in conjunction with current Human Tissue Act regulations.

<u>Additional research procedures</u>

CT scanning. In order to assess the effect of doxycycline on cystic change in the lungs and abdominal complications of LAM, a subset of patients will undergo CT scanning at the start and end of the study. Patients will consent specifically for this extra procedure.

Research blood sample. Patients who provide additional consent will undergo a blood test on the first visit and at the end of the study. This will be used for examination of gene and protein expression of relevance to LAM. In addition DNA will be removed and stored and genetic analysis performed on genes of relevance to LAM.

4. Data management

Statistical advice

Statistical advice and analysis of the study results will be performed by the trial statistician, Professor Sarah Lewis.

Statistical analysis of endpoints

Primary endpoint

The primary endpoint will be the difference in rate of decline of FEV_1 (ml/year) measured over 24 months.

All data will be analysed on an intention to treat basis in conjunction with the trial statistician Professor Sarah Lewis. The mean slope of regression lines for FEV_1 and FVC, plus change in DLCO, shuttle walk distance and quality of life in the doxycycline and placebo groups will be compared by parametric or non-parametric analysis as appropriate following analysis of the data for normality. In addition we will perform a *per protocol* analysis of mean rate of change of FEV_1/year. Time to composite safety endpoint will be analysed by Kaplan-Meier analysis, complications and adverse events by Chi Square test. Patients withdrawing from the study will continue to have measurements made where possible for intention to treat analysis. Missing values will be handled by multiple imputation.

Secondary endpoints

- The mean of regression lines for FVC measurements for each group (nine measurements per subject) will be compared by unpaired t-test with 0.05 taken as statistical significance. Patients will be analysed on an intention to treat basis with those withdrawing for any reason (including the combined safety criteria) will be analysed up to withdrawal.
- Change DLCO, shuttle walk distance and QoL in the doxycycline and placebo groups will be compared by parametric or non-parametric analysis. Data will be analysed for normality using the Kolmogorov-Smirnov method.

Toxicity and safety

- Time to composite safety endpoint by Caplan-Meier analysis.
- The number of LAM related complications (pneumothorax, chylous effusions, respiratory infections and angiomyolipoma bleeding) in each group will be analysed by Chi Square test.
- The number of doxycycline related side effects (related and unrelated) in each group will be analysed by Chi Square test.

Dosage

- We will present data on the relationship between doxycycline, serum and urine MMPs

5 Adverse Events Reporting

Adverse event reporting will be done in accordance with the principles of Good Clinical Practice and the requirements of the Medicines for Human Use (Clinical Trials) Regulations 2004

Definitions

Adverse event (AE) : Any untoward medical occurrence in a patient which does not necessarily have a causal relationship with this treatment.
This includes "any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study drug". This may include, for example, a cold, or an accident.

Adverse reaction to an investigational medicinal product (AR): all untoward and un-intended responses to an investigational medicinal product related to any dose administered.
All adverse events judged by either the reporting investigator or the chief investigator as having a reasonable causal relationship to a medicinal product qualify as adverse reactions.

Unexpected adverse reaction: an adverse reaction, the nature or severity of which is not consistent with the applicable product information i.e. the summary of product characteristics (SPC).

A serious adverse event or serious adverse reaction: any untoward medical occurrence or effect that at any dose fulfils at least one of the following criteria:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity

Or

- is a congenital anomaly/birth defect

Expected advents based on knowledge of TS and LAM

LAM

Pneumothorax
Chest infection
Chylothorax
Haemoptysis
Haemorrhage from AML

TSC
Pneumothorax
Chest infection
Chylothorax
Haemoptysis
Haemorrhage from AML
Seizures

Adverse event reporting procedure

Patients entered into the study must be encouraged from the outset of any study to contact the local principle investigator at the time of an event occurring. It is important that if patients are admitted to ward areas study staff are informed of the hospital admission as soon as possible. The appropriate study member should conduct study assessments, and ensure that all adverse events are identified for each patient as far as possible.

The Investigator should sign a written report of each serious adverse event forwarded to the research co-ordinating centre.

At each visit or study assessment adverse events that might have occurred since the previous visit or assessment should be elicited from the patient. For source documentation, verification of these events need to be detailed in the patients medical notes including the start dates (if known) of the onset of the event as well as the date the event stopped or changed, if applicable. Adverse events ongoing on completion of the study should be followed up as clinically indicated

<u>Procedure</u>

- Document the event on the adverse event form. Keep a copy of adverse event form (1) (see appendix 3) in the trial file within 7 days of first being aware of the adverse event.
- Document in a clear way as far as possible. For example, the patient may say that they ‘felt sick’. This can be interpreted in many ways: either they felt nauseated or they may have felt unwell, or they may even have been vomiting.
- Ask patient the date and start and stop time of event. If the patient cannot remember, then as near as possible.
- Document the action taken regarding study drug e.g. was the treatment dose reduced, or was study drug/treatment delayed etc.
- Document any treatment/medication given for the event, including the dates the treatment/medication was commenced and the date it was stopped/changed, if applicable.
- Document the event outcome.
- Document the seriousness (see the above definition of what is a serious event).
- Grade the adverse event using the National Cancer Institute’s Common Terminology Criteria for Adverse Events v3.0 (CTCAE)
- Document the causality. Does the adverse event have a reasonable suspected causal relationship with doxycycline?
- The relationship of adverse events to the products being studied should be determined according to the five categories classification below. Of the five, "possibly", "probably" and "definitely" related to an investigational medicinal product qualify as adverse reactions; "unlikely" and "not related" would not qualify as a reasonable causal relationship.

Not related - temporal relationship of the onset of the event, relative to administration of the product, is not reasonable or another cause can by itself explain the occurrence of the event.
Possibly related - temporal relationship of the onset of the event, relative to administration of the product, is reasonable but the event could have been due to another, equally likely cause.
Probably related - temporal relationship of the onset of the event, relative to administration of the product, is reasonable and the event is more likely explained by the product than by another cause.
Definitely related - temporal relationship of the onset of the event, relative to administration of the product, is reasonable and there is no other cause to explain the event.

- Document the expectedness (see the above definitions of an expected and unexpected event).
- Events ongoing at study completion should be followed up as clinically indicated.

Serious Adverse Events

- All adverse events/adverse drug reactions will be documented as above. However, if they come under the Serious Adverse Event definitions then the event will be classed as a serious adverse event:
- Inform the study co-ordinator by phone as soon as possible within 24-48 hours of the Investigator's knowledge of the event.
- Document the event on the adverse event form. Keep a copy of adverse event form in the local trial file and send a copy by post to the study co-ordinator within 7 days of first being aware of the adverse event.
- Respond promptly to requests for follow-up information from the Sponsor or other actions such as notification of the Ethics Committee if applicable. Store correspondence in the local trial site file.

Evaluation of adverse events by chief investigator

The study co-ordinator will inform the chief investigator of any adverse events, within 48 hours for an adverse event and within 24 hours of a serious adverse event. The chief investigator will evaluate the adverse event with respect to seriousness, causality and expectedness. The chief investigator will not overrule an assessment from a local investigator. In cases where there is a disagreement both opinions will be provided in subsequent reports.
The chief investigator will complete an adverse event form(II) (appendix 3).
A suspected unexpected serious adverse reactions (SUSAR) which is fatal or life-threatening will be reported to the MHRA and the Lead ethics committee within 4 days of the chief investigator becoming first aware of the reaction.

- A suspected unexpected serious adverse reactions (SUSAR) which is not fatal nor life-threatening will be reported to the MHRA, the Lead ethics committee and the

Data Monitoring and Safety Committee within 10 days of the chief investigator becoming first aware of the reaction.

- All documentation relating to adverse events will be stored in the main trial site file.

Annual report

The study co-ordinator will prepare an annual report of the study to the MHRA and the ethics committee. The report will provide a listing of serious adverse reactions

Monitoring

Monitoring of the study will be performed by the Nottingham Clinical Trial Support Unit.

6. **Doxycycline**

Posology and method of administration [19]

Dose. The usual dosage of Vibramycin for the treatment of acute infections in adults is 200mg on the first day (as a single dose or in divided doses) followed by a maintenance dose of 100mg/day. In the management of more severe infections, 200mg daily should be given throughout treatment.
Capsules and Dispersible Tablets are for oral administration only.
Vibramycin-D tablets are administered by drinking a suspension of the tablets in a small amount of water.
Vibramycin capsules should be administered with adequate amounts of fluid. This should be done in the sitting or standing position and well before retiring at night to reduce the risk of oesophageal irritation and ulceration. If gastric irritation occurs, it is recommended that Vibramycin be given with food or milk. Studies indicate that the absorption of Vibramycin is not notably influenced by simultaneous ingestion of food or milk.
Use in patients with impaired hepatic function. Vibramycin should be administered with caution to patients with hepatic impairment or those receiving potentially hepatotoxic drugs.
Abnormal hepatic function has been reported rarely and has been caused by both the oral and parenteral administration of tetracyclines, including doxycycline.
Use in patients with renal impairment. Studies to date have indicated that administration of Vibramycin at the usual recommended dose does not lead to accumulation of the antibiotic in patients with renal impairment.

Contraindications
Hypersensitivity. Persons who have shown hypersensitivity to doxycycline, any of its inert ingredients or to any of the tetracyclines.
Pregnancy. Vibramycin is contra-indicated in pregnancy. It appears that the risks associated with the use of tetracyclines during pregnancy are predominantly due to effects on teeth and skeletal development. (See above about use during tooth development).
Nursing mothers. Tetracyclines are excreted into milk and are therefore contra-indicated in nursing mothers. (Patients breast feeding will be excluded from the study).
Children. Doxycycline is contra-indicated in children under the age of 12 years. No children will be recruited to the study.

Special warnings and precautions for use
Use in patients with impaired hepatic function Vibramycin should be administered with caution to patients with hepatic impairment or those receiving potentially hepatotoxic drugs.
Abnormal hepatic function has been reported rarely and has been caused by both the oral and parenteral administration of tetracyclines, including doxycycline.
Use in patients with renal impairment. Excretion of doxycycline by the kidney is about 40%/72 hours in individuals with normal renal function. This percentage excretion may fall to a range as low as 1-5%/72 hours in individuals with severe renal insufficiency

(creatinine clearance below 10ml/min). Studies have shown no significant difference in the serum half-life of doxycycline in individuals with normal and severely impaired renal function. Haemodialysis does not alter the serum half-life of doxycycline. The anti-anabolic action of the tetracyclines may cause an increase in blood urea. Studies to date indicate that this anti-anabolic effect does not occur with the use of Vibramycin in patients with impaired renal function.

Photosensitivity. Photosensitivity manifested by an exaggerated sunburn reaction has been observed in some individuals taking tetracyclines, including doxycycline. Patients likely to be exposed to direct sunlight or ultraviolet light should be advised that this reaction can occur with tetracycline drugs and treatment should be discontinued at the first evidence of skin erythema.

Microbiological overgrowth The use of antibiotics may occasionally result in the overgrowth of non-susceptible organisms including Candida. If a resistant organism appears, the antibiotic should be discontinued and appropriate therapy instituted.

Pseudomembranous colitis has been reported with nearly all antibacterial agents, including doxycycline, and has ranged in severity from mild to life-threatening. It is important to consider this diagnosis in patients who present with diarrhoea subsequent to the administration of antibacterial agents.

Oesophagitis Instances of oesophagitis and oesophageal ulcerations have been reported in patients receiving capsule and tablet forms of drugs in the tetracycline class, including doxycycline. Most of these patients took medications immediately before going to bed or with inadequate amounts of fluid.

Bulging fontanelles in infants and benign intracranial hypertension in juveniles and adults have been reported in individuals receiving full therapeutic dosages. These conditions disappeared rapidly when the drug was discontinued.

Porphyria There have been rare reports of porphyria in patients receiving tetracyclines.

Beta-haemolytic streptococci infections Infections due to group A beta-haemolytic streptococci should be treated for at least 10 days.

Myasthenia gravis Due to a potential for weak neuromuscular blockade, care should be taken in administering tetracyclines to patients with myasthenia gravis.

Systemic lupus erythematosus Tetracyclines can cause exacerbation of SLE.

Methoxyflurane Caution is advised in administering tetracyclines with methoxyflurane.

<u>Interaction with other medicinal products and other forms of interaction.</u>

- The absorption of doxycycline may be impaired by concurrently administered antacids containing aluminium, calcium, magnesium or other drugs containing these cations; oral zinc, iron salts or bismuth preparations. Dosages should be maximally separated.
- Since bacteriostatic drugs may interfere with the bactericidal action of penicillin, it is advisable to avoid giving Vibramycin in conjunction with penicillin.
- There have been reports of prolonged prothrombin time in patients taking warfarin and doxycycline. Tetracyclines depress plasma prothrombin activity and reduced doses of concomitant anticoagulants may be necessary.
- The serum half-life of doxycycline may be shortened when patients are concurrently receiving barbiturates, carbamazepine or phenytoin. An increase in the daily dosage of Vibramycin should be considered.

- Alcohol may decrease the half-life of doxycycline.
- A few cases of pregnancy or breakthrough bleeding have been attributed to the concurrent use of tetracycline antibiotics with oral contraceptives.
- Doxycycline may increase the plasma concentration of cyclosporin. Co-administration should only be undertaken with appropriate monitoring.
- The concurrent use of tetracyclines and methoxyflurane has been reported to result in fatal renal toxicity.

Laboratory test interactions

False elevations of urinary catecholamine levels may occur due to interference with the fluorescence test.

Effects on ability to drive and use machines

The effect of doxycycline on the ability to drive or operate heavy machinery has not been studied. There is no evidence to suggest that doxycycline may affect these abilities.

Undesirable effects

The following adverse reactions have been observed in patients receiving tetracyclines, including doxycycline.

Autonomic nervous system. Flushing.

Body as a whole. Hypersensitivity reactions, including anaphylactic shock, anaphylaxis, anaphylactoid reaction, anaphylactoid purpura, hypotension, pericarditis, angioneurotic oedema, exacerbation of systemic lupus erythematosus, dyspnoea, serum sickness, peripheral oedema, tachycardia and urticaria.

Central and Peripheral nervous system. Headache. Bulging fontanelles in infants and benign intracranial hypertension in juveniles and adults have been reported in individuals receiving full therapeutic dosages of tetracyclines. In relation to benign intracranial hypertension, symptoms included blurring of vision, scotomata and diplopia. Permanent visual loss has been reported.

Gastro-intestinal. Gastro-intestinal symptoms are usually mild and seldom necessitate discontinuation of treatment. Abdominal pain, anorexia, nausea, vomiting, diarrhoea, dyspepsia and rarely dysphagia. Oesophagitis and oesophageal ulceration have been reported in patients receiving Vibramycin. A significant proportion of these occurred with the hyclate salt in the capsule form.

Hearing/Vestibular. Tinnitus.

Haematopoietic. Haemolytic anaemia, thrombocytopenia, neutropenia, porphyria, and eosinophilia have been reported with tetracyclines.

Liver/Biliary. Transient increases in liver function tests, hepatitis, jaundice, hepatic failure and pancreatitis have been reported rarely.

Musculo-Skeletal. Arthralgia and myalgia.

Skin. Rashes including maculopapular and erythematous rashes, exfoliative dermatitis, erythema multiforme, Steven-Johnson syndrome and toxic epidermal necrolysis. Photosensitivity skin reactions.

Superinfection. As with all antibiotics, overgrowth of non-susceptible organisms may cause candidiasis, glossitis, staphylococcal enterocolitis, pseudomembranous colitis (with Clostridium difficile overgrowth) and inflammatory lesions (with candidal overgrowth) in the anogenital region. Similarly there have been reports for products in the tetracycline class of stomatitis and vaginitis.
Urinary system. Increased blood urea.
Other. When given over prolonged periods, tetracyclines have been reported to produce brown-black microscopic discolouration of thyroid tissue. No abnormalities of thyroid function are known to occur.
Tetracyclines may cause discoloration of teeth and enamel hypoplasia, but usually only after long-term use.

Overdose
Acute overdosage with antibiotics is rare. In the event of overdosage discontinue medication. Gastric lavage plus appropriate supportive treatment is indicated. Dialysis does not alter serum half-life and thus would not be of benefit in treating cases of overdosage.

Pharmacodynamic properties
Vibramycin is primarily bacteriostatic and is believed to exert its antimicrobial effect by the inhibition of protein synthesis. Vibramycin is active against a wide range of Gram-positive and Gram-negative bacteria and certain other micro-organisms.

Pharmacokinetic properties
Tetracyclines are readily absorbed and are bound to plasma proteins in varying degrees. They are concentrated by the liver in the bile and excreted in the urine and faeces at high concentrations and in a biologically active form. Doxycycline is virtually completely absorbed after oral administration. Studies reported to date indicate that the absorption of doxycycline, unlike certain other tetracyclines, is not notably influenced by the ingestion of food or milk. Following a 200mg dose, normal adult volunteers averaged peak serum levels of 2.6 micrograms/ml of doxycycline at 2 hours decreasing to 1.45 micrograms/ml at 24 hours. Doxycycline has a high degree of lipid solubility and a low affinity for calcium. It is highly stable in normal human serum. Doxycycline will not degrade into an epianhydro form.

List of excipients
Vibramycin 50mg capsules: Maize Starch Ph.Eur., Lactose Ph.Eur., alginic acid, Magnesium Stearate NF, Sodium Lauryl Sulphate Ph Eur. In addition the capsule shell cap contains: Gelatin BP, titanium dioxide (E171), patent blue V (E131) and quinoline yellow (E104) and the body contains yellow iron oxide (E172), indigotine (E132) and titanium dioxide (E171).
Vibramycin D Dispersible tablets: Anhydrous Colloidal Silica Ph.Eur., Microcrystalline Cellulose Ph.Eur. and Magnesium Stearate Ph.Eur.

Shelf life
Vibramycin 50mg capsules 48 months.
Vibramycin D Dispersible tablets 48 months.

Store below 25°C.

Drug interactions

- Because tetracyclines have been shown to depress plasma prothrombin activity, patients who are on anticoagulant therapy may require downward adjustment of their anticoagulant dosage.
- Since bacteriostatic drugs may interfere with the bactericidal action of penicillin, it is advisable to avoid giving tetracyclines in conjunction with penicillin.
- Absorption of tetracyclines is impaired by antacids containing aluminum, calcium, or magnesium, and iron-containing preparations.
- Absorption of tetracycline is impaired by bismuth subsalicylate.
- Barbiturates, carbamazepine, and phenytoin decrease the half-life of doxycycline.
- The concurrent use of tetracycline and Penthrane (methoxyflurane) has been reported to result in fatal renal toxicity.
- Concurrent use of tetracycline may render oral contraceptives less effective.

Toxicity and interruption or discontinuation of treatment

Toxicity will be assessed by the National Cancer Institute's Common Terminology Criteria for Adverse Events v3.0 (CTCAE) (copy attached) (also available on http://ctep.cancer.gov/forms/CTCAEv3.pdf).

Expected adverse events related to doxycycline

Doxycycline is a widely used antibiotic for which serious side effects are rare. The drug is absorbed by the oral route and eliminated with a half-life of 18 hours, by faecal and renal excretion. Absorption is not significantly affected by the presence of food in the stomach. Doxycycline does not accumulate significantly in patients with renal function impairment. In the blood, doxycycline is 90 % protein-bound. The usual adult therapeutic dose is 100 to 200 mg/day.

Frequent adverse effects:

- Anorexia, nausea, vomiting, heart burn.
- Photosensitivity reactions upon exposure to direct sunlight, erythema or skin rashes. Skin and nail hyperpigmentation.
- Discoloration of teeth.
- Diarrhoea.

In addition a larger number of infrequent side effects have been reported.

Hematological. Haemolytic anaemia, thrombocytopenia, neutropenia and eosinophilia have all been reported (116-118). Elevation of pro-thrombin times can occur and for this reason those patients taking warfarin will be excluded from the study.

Central nervous system effects. Benign intracranial hypertension has been associated with doxycycline use which resolved when treatment was stopped. (119,120).

Endocrine/metabolic. Rare cases hypoglycaemia and lactic acidosis have been reported (121-123).

Gastrointestinal: Drug-induced oesophagitis occurs in about four percent of the population taking oral doxycycline (124)**.** This can be avoided by increasing water intake when swallowing the capsules. Nausea, vomiting anorexia, diarrhoea, glossitis and enterocolitis have also been reported. (125-139). Symptoms are often self-limiting. Antacids or sucralfate (136) will resolve most ulcers in several days to a week. Rarely, symptoms have lasted as long as 4 weeks after therapeutic intervention. Ulcers are more often associated with capsules than tablets, especially capsules that do not contain the enteric-coated formulation. Doxycycline should be taken with a full glass of water and the patient should remain in the upright position for at least thirty minutes. Preferably, it should be taken with food and not before going to sleep.

Renal. Doxycycline can be used safely in patients with renal disease as the drug can be eliminated almost completely through the gastrointestinal tract. Doxycycline may reduce the efficacy of oral contraceptives. Patients taking the oral contraceptive pill will be excluded from the study.

Skin: Photosensitivity reactions have been reported in about 8 to 11% of patients taking doxycycline (141-151). Patients will be advised to avoid direct sunlight and use sunscreens. Doxycycline should be discontinued if erythema occurs. Stevens-Johnson syndrome has been reported in three patients (152-154).

Alterations in gut microflora. Doxycycline in doses of 100 mg daily causes only moderate changes (suppression) in oropharyngeal and colonic microflora (155,156). Like other antibiotics, *Clostridium difficile* has been reported in some patients (157). In three studies, 40 mg / day of Doxycycline did not effect the composition or resistance of fecal or vaginal microflora over 9-months (158). Nor was there a difference in the number of side effects between patients treated with doxycycline or placebo for 9 months, (159).

Hypersensitivity. Hypersensitivity reactions including urticaria, angioneurotic oedema, anaphylaxis, purpura, and serum sickness are very rare (162,163).

Tooth discoloration. Permanent discoloration of the teeth has been associated with tetracyclines. This is most common in children under 8 (no children will be recruited to the study). Reversible tooth discoloration can occur in adults in short but more commonly long-term treatment (164).

<u>Management of adverse events associated with doxycycline</u>

Gastrointestinal. To reduce the risk of oesophagitis enteric-coated capsules will be used and patients will be advised to take doxycycline at meal times with a full glass of water. If these measures are unsuccessful, we will withdraw the drug and ask the patient to be evaluated by a gastroenterologist.

Cutaneous. Patients will be advised about the risk of photosensitivity, and to use good sun protection. For patients in whom a significant episode of tetracycline-induced

photosensitivity develops, treatment with doxycycline will be interrupted for a week to allow resolution of the skin reaction. Methods to protect the skin from exposure to light will be discussed with the patient, and doxycycline treatment will be resumed at the same dose. If this is unsuccessful in preventing further photosensitivity reactions, patients will be withdrawn from the study.

Dental. For patients in whom tooth discoloration develops, vigorous oral hygiene will be advised, along with regular follow-up with a dental technician for formal cleansing. Patients will be advised not to use chlorhexidine-containing mouthwashes, which can cause tooth discoloration. If this is unsuccessful in resolving tooth discoloration to the patient's satisfaction, the patient will be removed from the study.

Infectious. Doxycycline may cause moderate changes in oropharyngeal and colonic microflora. Non-antimicrobial doses of doxycycline do not appear to have an effect on the composition or resistance of faecal or vaginal microflora (167). It is possible that patients may become colonized with doxycycline resistant organisms. For patients in whom any form of yeast infection developed during the course of the study, doxycycline treatment may be interrupted to permit resolution of the infection, along with vigorous hygiene and anti-fungal treatment, followed by resumption of the study medication.

7 Project management

Trial personnel

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Study co-ordinator Medical research Fellow. To be appointed

Data monitoring and safety committee

Dr AM Freyer
Dr T Harrison
Dr I Le Jeune.

Patient contacts

A 24 hour emergency hotline for patients will be available. Additionally, between 9-5 all participants can contact the nominated physician at Nottingham or the study co-ordinator.

8 Administrative procedures

Recording of data and retention of documents

Local principal investigators will be responsible for completing all participant evaluation

forms and recording and reporting all adverse events as specified in the protocol. A copy of all completed participant evaluation forms will be kept locally and sent to the study co-ordinator.

All paper records will be kept in locked cabinets in the study centre. All electronically recorded data will be stored on a fire wall and password protected computer. We will comply with the requirements of the Data Protection Act. On entry participants will be assigned a participant number. All subsequent records (including scans) will be anonymised and the participant identified only by their participant number. All records will be kept for at least 10 years.

Monitoring procedures

A data monitoring committee of three people of appropriate expertise will provide independent monitoring of the study. They will be provided with safety data at six monthly intervals and review all adverse events. The committee will meet to review the study progress when 30 patients have been randomised and assess whether there are any safety reasons why the trial should not continue.
The study co-ordinator will have a house-keeping monitoring role ensuring that all data collected is consistent with adherence to the trial protocol, identifying any missing or inconsistent data, reviewing recruitment and ensuring that adverse event reporting is occurring as specified in the protocol.

Handling of study medication

Doxycycline and matched placebo will be obtained from Pfizer. Study drugs will be stored and dispensed from the clinical trial pharmacy at the Queens Medical Centre.

Publication of results

The results will be submitted to a peer reviewed journal. In addition presentations will be made at professional meetings and patient societies.

9 Consent

Patients on the UK LAM Register who have already given permission to be contacted about research studies, will be contacted by mail by the principal investigator. Potential participants will initially receive a information sheet giving information about the study with contact addresses and a response sheet for return by prepaid post. Those that express a potential interest in participating will be offered a face to face interview with one of the study doctors to assess eligibility, answer questions, obtain details of all physicians involved in their care and to obtain written consent prior to enrolment. Further patients will be recruited by word of mouth.

Individuals who will be taking consent
Dr. S. Johnson
Dr William Chang

Additional protocol information

Randomisation.
Randomisation will be handled by the Nottingham Clinical Trials Unit using a computer generated random sequence in a 1:1 ratio of drug to placebo. Randomisations will be performed by the study team via secure web based system hosted for the Nottingham Clinical Trials unit at the University of Nottingham.

Blinding
The study will be conducted in a double blind manner using an identical matched placebo and active doxycycline (purchased from Pfizer UK) and dispensed through the Nottingham Clinical Trials Pharmacy at the Queens Medical Centre.
Blinding is maintained for the patients, clinical investigators, including the study radiologist and laboratory staff performing MMP and other assays.

Study setting
All patients will be evaluated at the Queens medical centre either in the clinical trials facility or in the National Centre for LAM.

Appendix 1

Adverse events (1)

Patient number :

Nature of event:

Date of event
Start time
Stop time

Action taken re doxycycline

Treatment/ medication given
(include date started and stopped/changed)

Event outcome

Seriousness non-serious □ serious □

CTCAE description and grade (e.g. cardiac general, hypertension, grade 2)

Causality	Not related	□
	Unlikely	□
	Possibly related	□
	Probably related	□
	Definitely related	□

expectedness suspected □ unsuspected □

Time and date of notification of adverse event

Time and date study co-ordinator informed of adverse event

Adverse event form II
Seriousness non-serious □ serious □

Causality	Not related	□
	Unlikely	□
	Possibly related	□
	Probably related	□
	Definitely related	□

expectedness suspected □ unsuspected □

CTCAE description and grade (e.g. cardiac general, hypertension, grade 2)

Does this event need to be reported Yes □ No □

If yes

Time and date MHRA notified

Time and date ethics committee notified

Time and date Data and Safety committee notified

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SUPPLEMENTARY TABLES and FIGURES

	All patients (S.D.)	**Doxycycline** (S.D.)	**Placebo** (S.D.)
n	23	12	11
Lung function			
FEV_1 (l)	1.68 (0.77)	1.43 (0.81)	1.97 (0.64)
FVC (l)	3.10 (0.95)	3.06 (1.06)	3.16 (0.86)
TL_{CO} (kPa/min/ml)	4.38 (1.98)	3.81 (1.98)	5.00 (1.87)
TLC (l)	4.83 (1.20)	4.94 (1.46)	4.71 (0.90)

Table E1. Absolute values of lung function at baseline.

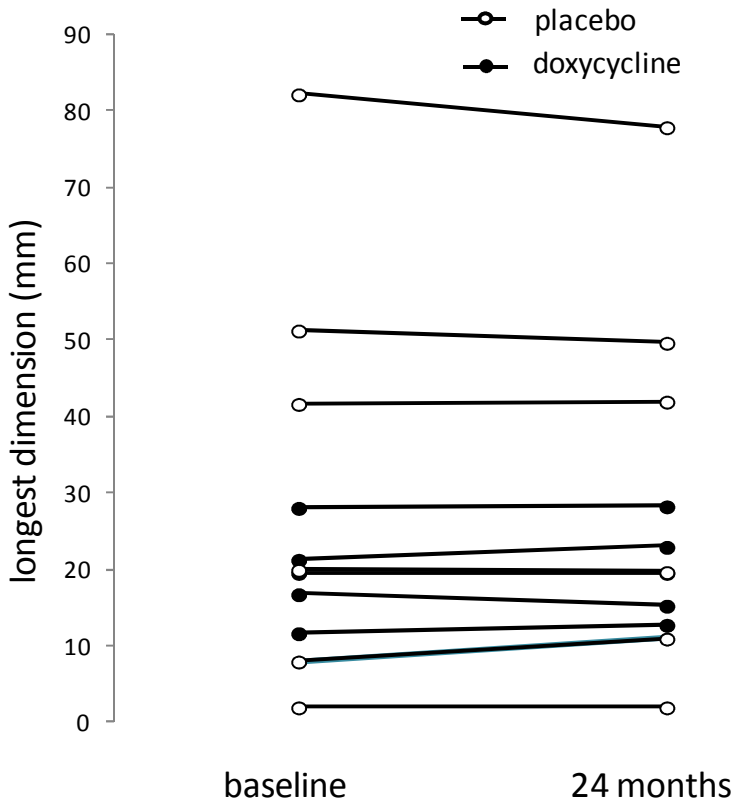

Figure E1. Effect of doxycycline on angiomyolipoma volume.
Twenty patients underwent CT scanning of the chest and abdomen at baseline. Thirteen had a follow scan at the end of the study period, these patients, 12 had an angiomyolipoma (11 solitary and multiple in one case). Angiomyolipoma size was measured by taking the longest dimension of the tumour from the CT image. In the patient with multiple angiomyolipoma only the largest was measured. No significant changes in the size of renal angiomyolipoma occurred in either group.

MMP Measurements in serum and urine.

In patients receiving doxycycline mean serum total MMP-9 was 272 ng/ml (SD 193) at baseline. After three months treatment with doxycycline ng/ml 100mg daily MMP-9 was 223 ng.ml (251), and 188 ng/ml (127) after a further 3 months of doxycycline 200mg daily. There was no significant difference in serum MMP-9 between groups over the 2 year period (p=0.49 figure E2). Total serum MMP-2 changed little during the study being 263 (SD 44) ng/ml at baseline, 249 (50) after doxycycline 100mg daily and 255 (48) after doxycycline 200mg daily (p=0.5, figure E3).

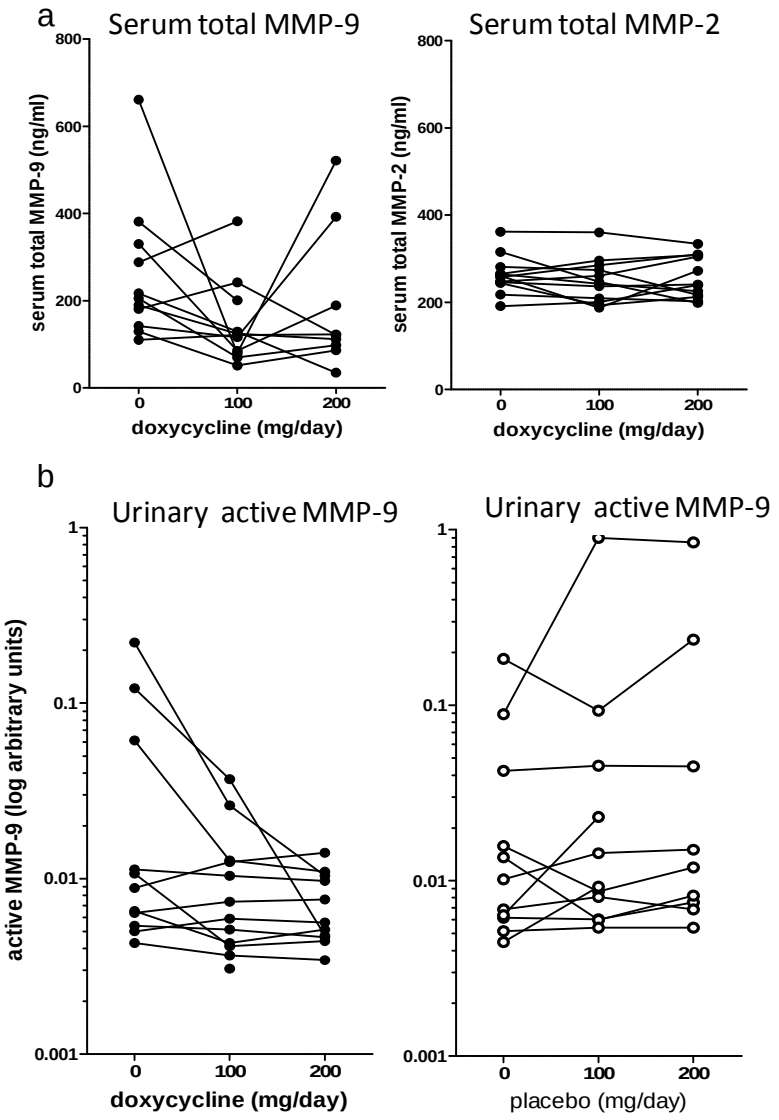

Figure E2. Effect of doxycycline dose and placebo on serum total and urine active MMP-9 in individual patients.
(a) Serum total MMP-9 was measured by specific ELISA. (b) Active MMP-9 were measured by gelatin zymography and quantitated by densitometry.

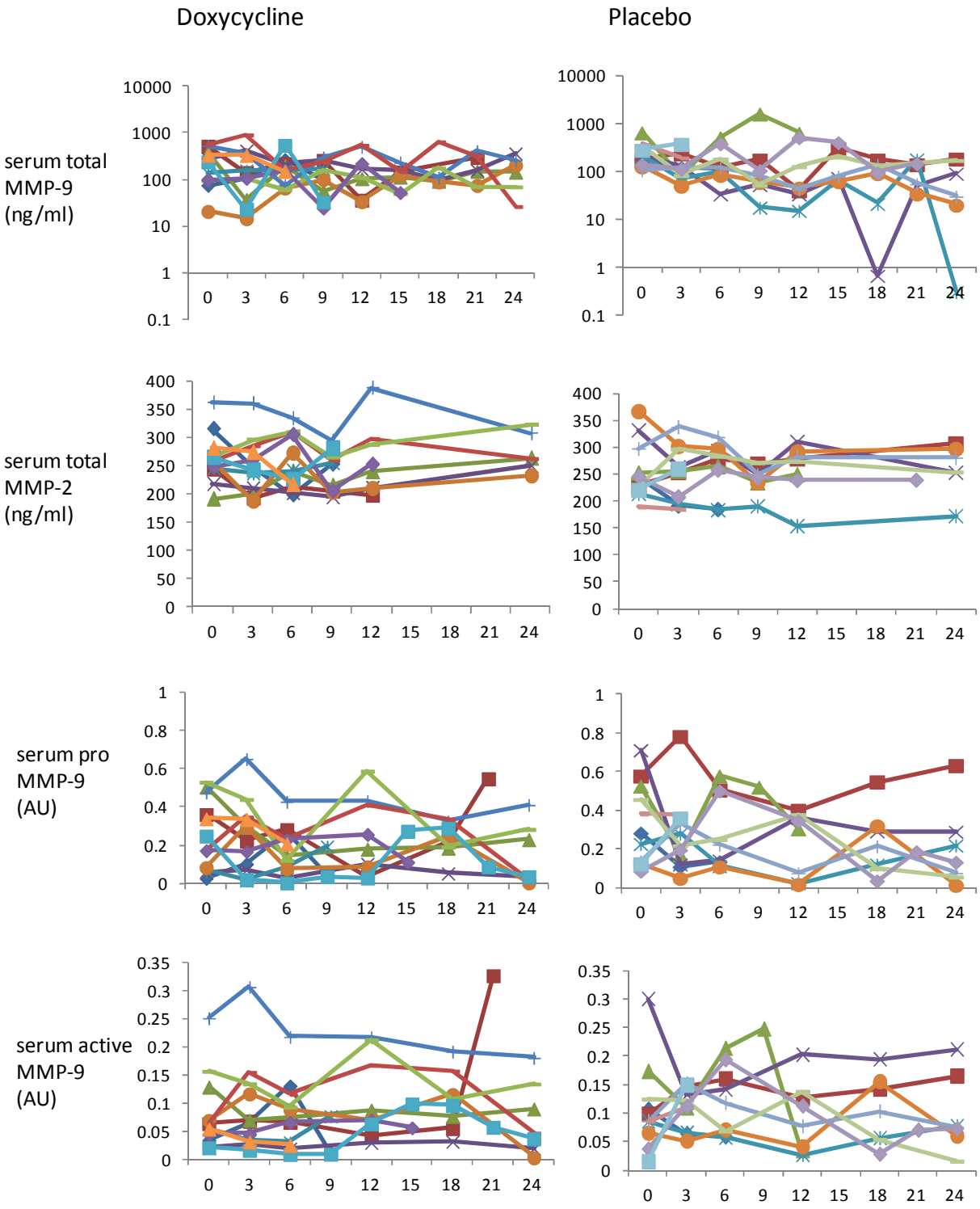

Figure E3. Serial serum MMP measurements for individual patients.
Serum total MMP-9 and -2 were measured by specific ELISA. Pro and active MMP-9 were measured by gelatin zymography and quantitated by densitometry. Measurements of MMP-2 were not made at 15, 18 or 21 months.

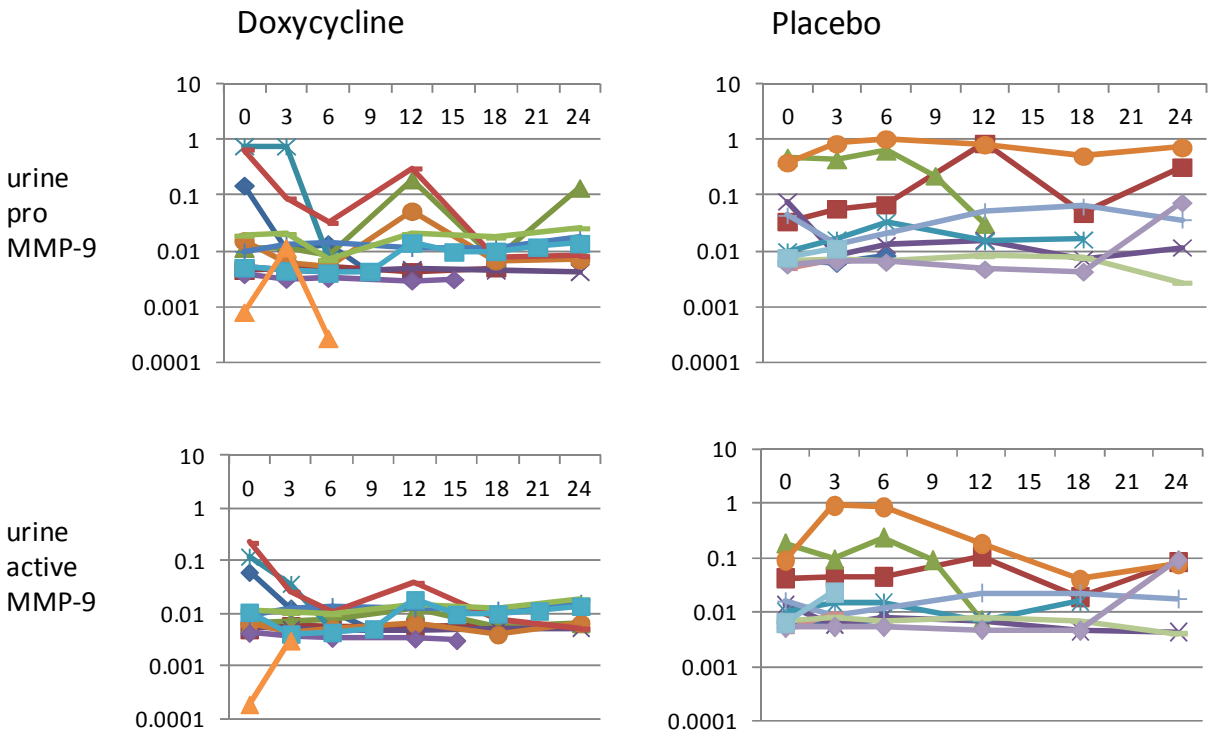

Figure E4. Serial urine MMP-9 measurements for individual patients.
Pro and active MMP-9 were measured by gelatin zymography and quantitated by densitometry.