APPENDIX

Section 1 - Additional methodological details

Urinary matrix metalloproteinase (MMP)-2 levels, serum MMP-9 levels, and serum VEGF-D levels were measured by ELISA (R&D System, Inc., Minneapolis, MN, USA), according to the specific antibody binding.

Samples were centrifuged at 1,500 rpm for 10 min and stored at −80°C. The MMP levels were determined by optical density comparison in an ELISA reader (Power Wave; Bio-Tek Instruments Inc., Winooski, VT, USA) with a 450-nm filter. The detection limit of the assay was 15.6 pg/mL for MMP-9, 156.2 pg/mL for MMP-2, and 15.6 pg/mL for VEGF-D.

Section 2 - Additional results

Table A1 - Baseline characteristics of the 31 lymphangioleiomyomatosis patients who completed the study, as well as pulmonary function test results and biomarkers in those patients.[a]

Variable	Result
Age, years	43 ± 8
Tuberous sclerosis[b]	4 (13)
Biopsy-proven diagnosis[b]	29 (94)
Hormonal blockade[b]	
GnRH analogue	14 (45)
Oophorectomy	4 (13)
Menopause	4 (13)
Pulmonary function test	
FVC, L	3.2 ± 0.6
FVC, % of predicted	92 ±1 4
FEV_1, L	2.2 ± 0.7
FEV_1, % of predicted	79 ± 23
FEV_1/FVC ratio	0.7 ± 0.2
TLC, L	5.0 ± 0.8
TLC, % of predicted	103 ± 14
RV, L	1.8 ± 0.6
RV, % of predicted	130 ± 47
RV/TLC ratio	0.36 ± 0.08
DLCO, mL/min/mmHg	17.0 ± 6.8
DLCO, % of predicted	65 ± 25
Six-minute walk test	
Distance, m	490 ± 109
Distance, % of predicted	90 ± 20
Baseline SpO_2, %[c]	96 (95-98)
Minimum SpO_2, %[c]	94 (87-95)
Biomarkers[c,d]	
Serum MMP-9, ng/mL	933 (730-1,202)
Urinary MMP-9, pg/mL	10,487 (4,565-20,963)
Serum MMP-2, pg/mL	0 (0-833)
VEGF-D, pg/mL	821 (407-2,113)

GnRH: gonadotropin-releasing hormone; minimum SpO_2: minimum SpO_2 sustained for 10 s; and MMP: matrix metalloproteinase. [a]Values expressed as mean ± SD, except where otherwise indicated. [b]Values expressed as n (%). [c]Values expressed as median (interquartile range). [d]Two patients provided no blood or urine samples.

Table A2 - Comparison between the lymphangioleiomyomatosis patients in the doxycycline-responder group and those in the doxycycline-nonresponder group in terms of baseline markers of functional response to doxycycline.[a]

Variable	Group		p
	doxy-R	doxy-NR	
	(n = 13)	(n = 18)	
FVC, L	3 ± 0.3	3.2 ± 0.7	0.120
FVC, % of predicted	90 ± 13	94 ± 15	0.520
FEV_1/FVC ratio	0.77 ± 0.10	0.65 ± 0.20	0.031
TLC, L	4.6 ± 0.5	5.3 ± 0.8	0.006
TLC, % of predicted	98 ± 13	107 ± 13	0.720
RV, L	1.6 ± 0.5	2.0 ± 0.6	0.087
RV, % of predicted	117 ± 43	140 ± 49	0.201
RV/TLC ratio	0.34 ± 0.08	0.37 ± 0.08	0.382
DLCO, mL/min/mmHg	18.5 ± 6.0	15.9 ± 7.5	0.297
DLCO, % of predicted	72 ± 20	60 ± 27	0.198

Doxy-R: doxycycline-responder; and doxy-NR: doxycycline-nonresponder. [a]Values expressed as mean ± SD.

Table A3 - Comparison between the lymphangioleiomyomatosis patients in the doxycycline-responder group and those in the doxycycline-nonresponder group in terms of the prevalence of hormonal blockade therapy before the treatment with doxycycline.[a]

Hormonal blockade	Groups				Total		p*
	doxy-R		doxy-NR				
	n	%	n	%	n	%	
No	3	33	6	67	9	29	0.696
Yes	10	45	12	55	22	71	
GnRH analogue	6		8				
Oophorectomy	2		2				
Menopause	2		2				

Doxy-R: doxycycline-responder; doxy-NR: doxycycline-nonresponder; and GnRH: gonadotropin-releasing hormone. [a]Values expressed as n (%). *Fisher's exact test.

Table A4 - Doxycycline-related adverse events during the study period.[a]

Adverse event	Baseline	Post-treatment
	(n = 41)	(n = 31)
Nausea	10 (24)	6 (19)
Epigastric pain	20 (49)	14 (45)
Diarrhea	8 (20)	5 (16)
Vomiting	1(2)	0 (0)
Itching	2 (5)	2 (6)
Chest pain	1 (2)	1 (3)
Photosensitivity reaction	1 (2)	1 (3)
Maculopapular rash	2 (5)	2 (6)

[a]Values expressed as n (%).

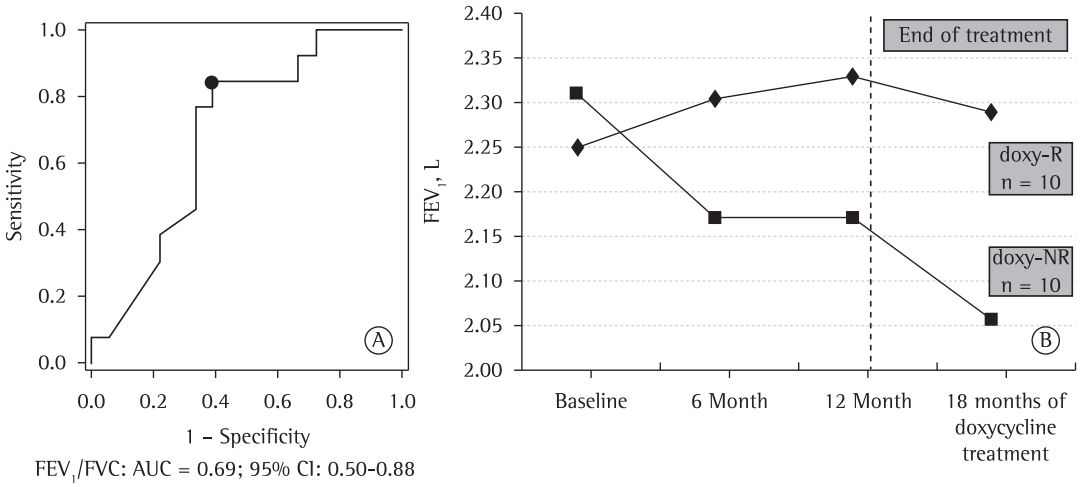

Figure A1 - ROC curve and FEV_1 follow-up. In A, ROC curve for predicting the response to doxycycline treatment. The solid dot indicates the cut-off point for the FEV_1/FVC ratio that was most accurate in predicting the response to treatment (0.71). In B, FEV_1 follow-up of 20 lymphangioleiomyomatosis patients who continued to use doxycycline for a median time of 18 months after the end of the 12-month study period. The patients in the doxycycline-responder (doxy-R) group showed a slight reduction in FEV_1 (from 2.33 L to 2.29 L), whereas those in the doxycycline-nonresponder (doxy-NR) group showed a sizeable decline in FEV_1 (from 2.17 L to 2.06 L). AUC: area under the curve. The doxy-R group comprised the lymphangioleiomyomatosis patients who responded to treatment with doxycycline, as evidenced by increased or stable FEV_1 at doxycycline treatment month 12 in comparison with FEV_1 at baseline. The doxy-NR group comprised the lymphangioleiomyomatosis patients who did not respond to treatment with doxycycline, as evidenced by decreased FEV_1 at doxycycline treatment month 12 in comparison with FEV_1 at baseline.