Unexpected sirolimus-stimulated airway hyperreactivity in lymphangioleiomyomatosis

Received: 11 May 2023
Accepted: 20 May 2023

To the Editor:

Lymphangioleiomyomatosis (LAM) is a multisystem disease affecting primarily women, characterised in the lung by proliferation of LAM cells, abnormal smooth muscle-like cells with dysfunctional tuberous sclerosis complex genes. This dysfunction results in activation of mechanistic target of rapamycin (mTOR), leading to LAM cell proliferation. Sirolimus (rapamycin) is the only United States Food and Drug Administration-approved treatment for pulmonary LAM, resulting in decreased LAM cell growth/size and stabilised lung function [1].

LAM is characterised by lung destruction, with cysts lined by nodules containing LAM cells and immune cells. Regions of the basement membrane are disrupted, with loss of lung parenchyma and gas exchange impairment [1]. LAM patients may exhibit partially reversible airflow obstruction [2], and patients with more LAM nodules have higher frequencies of bronchodilator response (BDR), suggesting that airflow obstruction results from an increase in airways resistance due to the proliferation of LAM cells surrounding the airways [2]. Patients with advanced stage LAM had bronchi showing LAM cell infiltration [3]. A greater frequency of BDR was a clinical marker of worse lung function and a greater rate of decline in forced expiratory volume in 1 s (FEV_1) and diffusing capacity of the lung for carbon monoxide (D_{LCO}) in LAM patients without sirolimus treatment [2].

Airway hyperresponsiveness may stem from increased airway smooth muscle (*i.e.* LAM cell growth and proliferation) [2]; an increase in contractile mediators (*i.e.* mast cell products [4]); and/or increases in intracellular calcium that enhance airway smooth muscle contractility (*i.e.* ryanodine receptor activation) among other factors [5, 6]. Since sirolimus stabilises LAM disease, we predicted that BDR frequency in patients receiving sirolimus would decrease as compared to BDR frequency of patients pre-treatment, and thus be indicative of treatment success.

To test this idea, we examined the BDR of 133 patients (105 with visits both before and during sirolimus treatment, 28 with visits only during treatment), with 1528 visits. Patients were diagnosed based on clinical, radiological, physiological and pathological criteria [7, 8], with written informed consent from the National Institutes of Health Institutional Review Board protocols 95-H-0186 and 96-H-0100. BDR was defined as an albuterol-induced increase of FEV_1 of $\geqslant$12% over baseline. As 48.0% of visits on sirolimus had a baseline FEV_1 of <1.5 L, this definition of BDR using a percentage-based difference over baseline as opposed to a volume-based cut-off is warranted, as clinically relevant BDR may be missed in patients with low baseline FEV_1 who cannot produce large changes in volume [9].

Unexpectedly, a BDR was seen at 34.9% (310 out of 887) of visits during treatment as compared to 25% (160 out of 641) of pre-treatment visits ($p<0.001$). To determine whether this result was due to the association of worse lung function and BDR [2], we defined severe pulmonary disease as having FEV_1 or D_{LCO} $\leqslant$40% predicted, with 14.4% of pre-treatment visits and 36.4% of visits during treatment falling into this category. Regardless of sirolimus treatment, the frequency of BDR was increased in those with severe disease as compared to those with normal/mild/moderate disease ($p<0.001$) (figure 1a). However, in those with normal/mild/moderate disease, there was an increase in BDR frequency during sirolimus treatment

Shareable abstract (@ERSpublications)
Airway hyperreactivity increases with sirolimus treatment in LAM patients with moderate disease, despite LAM disease stabilisation. A possible explanation for the increase in airway hyperreactivity is stimulation of the ryanodine receptor by sirolimus. https://bit.ly/3OcsggU

Cite this article as: Steagall WK, Stylianou M, Pacheco-Rodriguez G, *et al*. Unexpected sirolimus-stimulated airway hyperreactivity in lymphangioleiomyomatosis. *ERJ Open Res* 2023; 9: 00305-2023 [DOI: 10.1183/23120541.00305-2023].

a)

	Pre-treatment		During sirolimus treatment	
BDR	Normal/mild/moderate lung disease Visits n (%)	Severe lung disease Visits n (%)	Normal/mild/moderate lung disease Visits n (%)	Severe lung disease Visits n (%)
No	431 (78.6)	50 (54.3)	405 (71.8)	172 (53.3)
Yes	118 (21.4)	42 (45.7)#	159 (28.2)¶	151 (46.7)#
BDR	Patients n (%)		Patients n (%)	
Never	15 (33.3)		23 (31.5)	
<50%	19 (42.2)		27 (37.0)	
≥50%	11 (24.4)		23 (31.5)	
	Average % predicted			
BDR	FEV_1	D_{LCO}	FEV_1	D_{LCO}
Never	84.4	75.8	56.5	46.9
<50%	70.7+	64.0+	55.2	47.2
≥50%	72.4+	67.5	51.9	43.5
	Rate of change (% predicted per year)			
BDR	FEV_1	D_{LCO}	FEV_1	D_{LCO}
Never	−2.01	−1.94	−0.55	−0.71
<50%	−2.37	−2.45	−0.98	−1.33
≥50%	−2.16	−2.41	0.07§	−0.50§

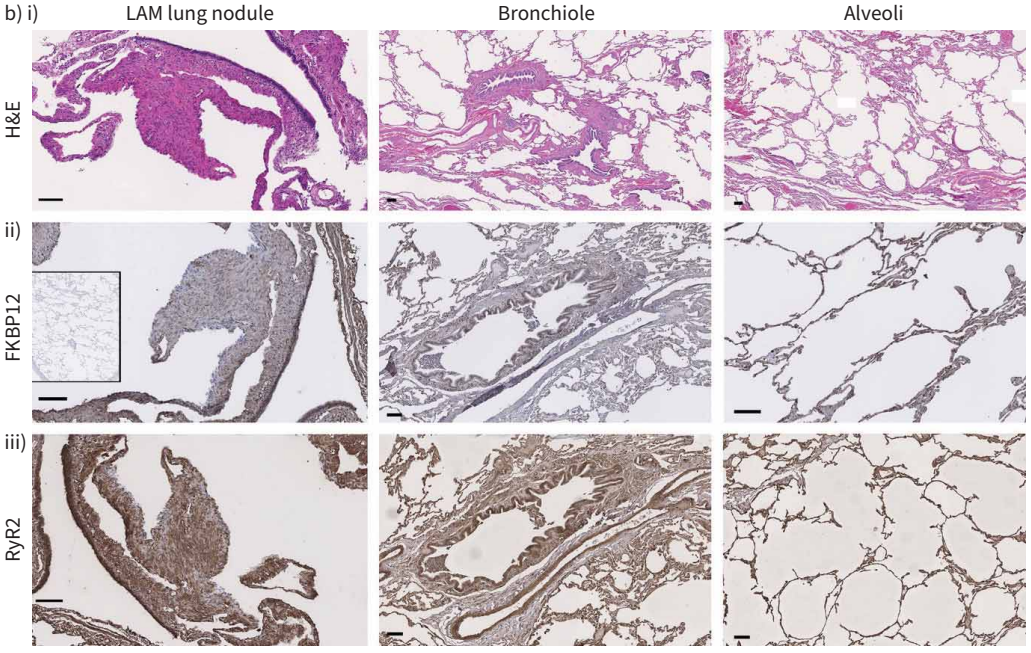

FIGURE 1 a) Frequency of bronchodilator response (BDR) seen at visits either pre-treatment or during sirolimus treatment for patients with normal/mild/moderate lung disease or severe lung disease, with severe disease defined as either forced expiratory volume in 1 s (FEV_1) or diffusing capacity of the lung for carbon monoxide (D_{LCO}) ⩽40% pred, and BDR frequency, average percent predicted pulmonary function values and rates of change in percent predicted FEV_1 or D_{LCO} for patients with at least five visits before and during treatment. Flow rates were measured before and after nebulisation with 2.5 mg albuterol. An increase of FEV_1 of ⩾12% over baseline was considered a positive response. Repeated measurements of pulmonary function tests were analysed using mixed-effects models. Analysis of each variable (FEV_1, D_{LCO}), was adjusted for its initial value, time of visit and sirolimus treatment. Data analysis was performed using SAS 9.3. 133 patients: 132 female, one male; 10 Asian, three African-American, one Hawaiian/Pacific Islander, four multiple race (two Hispanic/Latino), five unknown race (four Hispanic/Latino), 110 Caucasian (six Hispanic/Latino); 110 sporadic lymphangioleiomyomatosis (LAM), 22 LAM/tuberous sclerosis complex (TSC), one LAM with questionable TSC status; mean±SEM age at start of study 42.0±0.8 years; mean±SEM age at start of sirolimus treatment 46.8 ±0.9 years; mean±SEM FEV_1 and D_{LCO} at the first pre-treatment visit 79.0±2.3% pred and 70.3±2.2% pred,

respectively; mean±SEM FEV_1 and D_{LCO} at the start of sirolimus treatment 67.7±2.0% pred and 59.5±1.9% pred, respectively. b) FKBP12 and ryanodine receptor type 2 (RyR2) are present in lymphangioleiomyomatosis (LAM) lung nodules and bronchioles. Images are representative of results from three patients. i) Tissue architecture of tissue sections from explanted lungs of patients with LAM (haematoxylin and eosin (H&E)). ii) Proliferative LAM cells immunoreactive with anti-FKBP12 polyclonal antibodies (Invitrogen). Inset is the negative control for antibodies using a nonspecific rabbit antibody. Epithelial cells from bronchioles are also immunoreactive to anti-FKBP12 antibodies. Alveoli present a reactivity to anti-FKBP12 as well. iii) RyR2 is present in LAM lung nodules, bronchioles, and endothelial cells. Alveoli reacted to the anti-RyR2 polyclonal antibodies (Invitrogen). Tissue sections were stained using an antigen retrieval procedure and peroxidase/3,3′-diaminobenzidine method. Scale bars=100 μm. #: BDR frequency increases with severe disease regardless of sirolimus treatment (p<0.001 for both pre-treatment normal/mild/moderate disease *versus* severe lung disease and during treatment normal/mild/moderate disease *versus* severe lung disease); ¶: patients with normal/mild/moderate lung disease show an increase in BDR frequency with sirolimus treatment (p=0.013); +: p<0.005 compared to the "never" category; §: p<0.001 comparing <50% to ⩾50%.

(27.9%) as compared to pre-treatment (21.4%) (p=0.013) that was not seen in those with severe disease (46.7% during treatment *versus* 45.7% pre-treatment). This increase in BDR frequency in those patients with normal/mild/moderate disease during treatment is opposite to our prediction that sirolimus treatment would decrease BDR frequency.

To control for the inherent variability in BDR, we examined BDR in patients over multiple visits, grouping them as never having a BDR, having a BDR <50% of the time, or having a BDR ⩾50% of the time, followed for at least five visits. As determined previously, patients without sirolimus treatment were more likely to have BDR when they had worse lung function and showed a trend towards faster decline in pulmonary function as compared to those without BDR [2]. For patients during sirolimus treatment, there was a trend towards worse pulmonary function values in those patients with BDR. Interestingly, patients during sirolimus treatment who showed a BDR ⩾50% of the time had significantly slower rates of decline of pulmonary function than those with BDR <50% of the time, suggesting that those with more stable disease show more frequent BDR during sirolimus treatment than those with declining disease (figure 1a).

When we examine BDR over time, we find that, pre-treatment, the longer time since the first visit, the higher the probability of BDR (p=0.002), suggesting that BDR frequency increases as disease worsens, as expected. Surprisingly, during treatment, increasing the time on sirolimus decreases the probability of BDR (p<0.001), despite more severe disease. This may be due to the inability of the destroyed lung to support an increase in FEV_1 of 12% upon β-agonist stimulation. Examination of transplanted lungs by *ex vivo* computed tomography indicated that LAM lungs have at least a three-fold reduction in airway number, with collapse of airways due to cysts and filling of airways with exudate [10].

The increase in BDR frequency in patients with normal/mild/moderate disease during sirolimus treatment as compared to the BDR frequency in the same patients before treatment is unexpected, as treatment would be expected to slow LAM disease, relieving airway obstruction [11]. A possible explanation may be activation of the ryanodine receptor [12], a channel responsible for changes in calcium concentrations in the cell. It is stabilised in a closed position by FKBP12. Sirolimus binds to FKBP12 and removes it from the ryanodine receptor, resulting in its activation. Stimulation of the ryanodine receptor in normal cells by sirolimus may explain some side effects seen with sirolimus treatment [1], since the ryanodine receptor is linked to airway hyperreactivity, lymphoedema and hypertension [12–14], as airway smooth muscle, lymphatic, and endothelial cells express ryanodine receptors. We immunostained LAM lung tissue from three patients with polyclonal antibodies to FKBP12 and ryanodine receptor type 2 (RyR2) using an antigen retrieval procedure and peroxidase/3,3′-diaminobenzidine method (figure 1b). Proliferative LAM cells, epithelial cells from bronchioles, and alveoli were immunoreactive with anti-FKBP12 antibodies. RyR2 was present in alveoli, LAM lung nodules, bronchioles and endothelial cells. Interestingly, RyR2 was detected in LAM cells, in addition to its expected presence in normal cells. The effect of ryanodine receptor activation in LAM cells warrants further investigation.

In patients before treatment with sirolimus, mTOR would be active and the FKBP12/ryanodine receptor complex would be stabilised in a closed state, suggesting that airway hyperreactivity is due to LAM cell hypertrophy/hyperplasia or other factors, such as mast cell recruitment [4]. When patients are treated with sirolimus, FKBP12/sirolimus/mTOR would be inactive and the ryanodine receptor would be in an open

state, suggesting that airway hyperreactivity is due to increases in intracellular free calcium ions in normal airway cells. Thus, in LAM, the clinical marker of a BDR during sirolimus treatment may not be indicative of worsening disease, but may be indicative of adverse events due to ryanodine receptor activation in nontargeted normal cells. Ryanodine receptor activation by sirolimus explains airway hyperreactivity seen with treatment *via* effects on normal cells, while also allowing for LAM disease stabilisation by sirolimus. Patients with BDRs may be treated with steroids; in the case of those with sirolimus treatment, perhaps therapy targeting the ryanodine receptor may be a better choice.

Wendy K. Steagall[1], Mario Stylianou[2], Gustavo Pacheco-Rodriguez[1], Zu Xi Yu[3] and Joel Moss[1]

[1]Pulmonary Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA. [2]Office of Biostatistics Research, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA. [3]Pathology Facility, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD, USA.

Corresponding author: Joel Moss (mossj@nhlbi.nih.gov)

Submitted article, peer reviewed.

Conflict of interest: All authors declare no conflicts of interest and no competing interests.

Support statement: The study was supported by the Intramural Research Program, National Institutes of Health, National Heart, Lung, and Blood Institute.

References

1 McCarthy C, Gupta N, Johnson SR, *et al.* Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. *Lancet Respir Med* 2021; 9: 1313–1327.
2 Taveira-DaSilva AM, Steagall WK, Rabel A, *et al.* Reversible airflow obstruction in lymphangioleiomyomatosis. *Chest* 2009; 136: 1596–1603.
3 Hayashi T, Kumasaka T, Mitani K, *et al.* Bronchial involvement in advanced stage lymphangioleiomyomatosis: histopathologic and molecular analyses. *Hum Pathol* 2016; 50: 34–42.
4 Babaei-Jadidi R, Dongre A, Miller S, *et al.* Mast-cell tryptase release contributes to disease progression in lymphangioleiomyomatosis. *Am J Respir Crit Care Med* 2021; 204: 431–444.
5 Doeing DC, Solway J. Airway smooth muscle in the pathophysiology and treatment of asthma. *J Appl Physiol* 2013; 114: 834–843.
6 Parameswaran K, Janssen LJ, O'Byrne PM. Airway hyperresponsiveness and calcium handling by smooth muscle: a 'deeper look'. *Chest* 2002; 121: 621–624.
7 Johnson SR, Cordier JF, Lazor R, *et al.* European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. *Eur Respir J* 2010; 35: 14–26.
8 McCormack FX, Gupta N, Finlay GR, *et al.* Official American Thoracic Society/Japanese Respiratory Society clinical practice guidelines: lymphangioleiomyomatosis diagnosis and management. *Am J Respir Crit Care Med* 2016; 194: 748–761.
9 Hansen JE, Dilektasli AG, Porszasz J, *et al.* A new bronchodilator response grading strategy identifies distinct patient populations. *Ann Am Thorac Soc* 2019; 16: 1504–1517.
10 Verleden SE, Vanstapel A, De Sadeleer L, *et al.* Quantitative analysis of airway obstruction in lymphangioleiomyomatosis. *Eur Respir J* 2020; 56: 1901965.
11 Argula RG, Kokosi M, Lo P, *et al.* A novel quantitative computed tomographic analysis suggests how sirolimus stabilizes progressive air trapping in lymphangioleiomyomatosis. *Ann Am Thorac Soc* 2016; 13: 342–349.
12 Du Y, Zhao J, Li X, *et al.* Dissociation of FK506-binding protein 12.6 kD from ryanodine receptor in bronchial smooth muscle cells in airway hyperresponsiveness in asthma. *Am J Respir Cell Mol Biol* 2014; 50: 398–408.
13 Pal S, Rahman J, Mu S, *et al.* Drug-related lymphedema: mysteries, mechanisms, and potential therapies. *Front Pharmacol* 2022; 13: 850586.
14 Kaßmann M, Szijártó IA, García-Prieto CF, *et al.* Role of ryanodine type 2 receptors in elementary Ca^{2+} signaling in arteries and vascular adaptive responses. *J Am Heart Assoc* 2019; 8: e010090.