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# Lymphangioleiomyomatosis

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**CONTENTS**



**Abstract**

This issue's Filling the Gaps in Drug Therapy focuses on lymphangioleiomyomatosis, a rare, multiorgan disease of unknown etiology that primarily affects women. Pulmonary symptoms, as well as abdominal cysts (lymphangioleiomyomas), are hallmarks of this potentially fatal condition. Treatment of lymphangioleiomyomatosis is challenging, as currently available options are not curative and only delay disease progression. This article summarizes current and future therapies, emphasizing potential drug targets that have arisen from the latest research in this area.

## Introduction

Lymphangioleiomyomatosis (LAM) is a rare, potentially fatal disease that occurs predominantly in women and affects multiple organs, particularly the lungs, kidneys and abdominal and pelvic lymphatics. Pulmonary symptoms may gradually progress to respiratory failure requiring lung transplantation. Although its exact etiology is unknown, about one-third of LAM cases present concomitantly with tuberous sclerosis complex (TSC), an autosomal dominant syndrome involving germline mutations in the tumor suppressor genes *TSC1* and *TSC2*. However, there is also a sporadic form of LAM that occurs independently of TSC. The prevalence of sporadic LAM has been estimated to be approximately 3-5 per million people, whereas TSC-associated LAM (TSC-LAM) is much more common (about 250,000 patients worldwide) (1).

Hormonal dependence has been suggested in LAM etiopathogenesis, as sporadic LAM occurs exclusively in women of childbearing age and only very few cases of TSC-LAM have been reported in men. Recent data from a large LAM registry established by the National Heart, Lung and Blood Institute (NHLBI) that enrolled 243 subjects, all of them women, found that the age range of affected patients was broader than in previous studies and over one-third of the subjects were postmenopausal (2).

Usually, LAM onset occurs around 34-39 years of age and is diagnosed by pulmonary manifestations, primarily spontaneous pneumothorax, although dyspnea is also common (2, 3). Other respiratory symptoms, such as cough, hemoptysis or chyloptysis (chylous pleural effusion), are less frequent. Abdominal lymphadenopathy and the presence of cystic lymphatic masses or lymphangioleiomyomas are other disease hallmarks. Lymphangioleiomyomas can cause nausea, bloating, abdominal distension, peripheral edema and urinary symptoms, which may worsen during the day as cyst size increases in the afternoon, likely due to lymph accumulation in lower limbs (3). Another common abdominal finding are angiomyolipomas, benign tumors of renal localization that occur in about half of LAM patients. Angiomyolipomas are frequently small and asymptomatic, but they can reach large proportions (about 20 cm) and completely replace normal kidney parenchyma. Cysts exceeding 4 cm in diameter should be monitored as life-threatening bleeding can occur (3).

Patient survival has been estimated at approximately 10 years after the onset of symptoms, although a recent analysis of the U.K. national LAM database reported that some patients were still alive 20 years after disease onset (4). In this study, smoking, pregnancy and progesterone treatment appeared to be associated with a more severe prognosis, although prospective trials are needed to validate these findings (4).

## Genetics

The fact that pulmonary lesions in both sporadic and TSC-associated LAM share almost identical characteristics suggested a common pathogenic mechanism for both disease types. As mentioned earlier, about one-third of LAM patients may present with TSC, an autosomal dominant disorder featuring LAM-like lung and kidney manifestations due to inactivating mutations of the tumor sup-

pressor gene *TSC1* or *TSC2.* Hamartin and tuberin are *TSC1* and *TSC2* gene products, respectively, which form a complex that plays a central role in cell growth signaling pathways. In the absence of growth factors, hamartin/tuberin (TSC1/TSC2) complexes suppress the activity of the mammalian target of rapamycin (mTOR), which controls cell growth and proliferation via the synthesis of new proteins. The hamartin/tuberin complex exerts strong GTPase-activating (GAP) activity on the Rheb protein, a GTPase of the Ras superfamily and key regulator of the mTOR activation state, thus favoring its inactive Rheb-GDP-bound configuration. However, upon extracellular growth factor binding, tuberin is phosphorylated and the complex loses its Rheb-GAP activity, causing Rheb-GTP levels to rise and thus prompting mTOR activation and subsequent cell growth and proliferation. In TSC, the absence of a functional TSC1/TSC2 complex leads to constitutive activation of mTOR signaling, resulting in uncontrolled protein synthesis and aberrant cell growth (5).

However, sporadic LAM has also been related to mutations in *TSC2.* Examination of angiomyolipomas and pulmonary lesions from sporadic LAM patients provided evidence for the existence of the same *TSC2* mutation types in both affected tissues, which were not present in normal kidney and lung samples (6), confirming the somatic nature of these mutations. In contrast, tuberous sclerosis features *TSC2* germline mutations that can be found in both tumor and normal tissues (7). Loss of *TSC2* heterozygosity for the wild-type allele was also found in angiomyolipomas and pulmonary cells from sporadic LAM patients, indicating that inactivation of both *TSC2* alleles occurs in LAM, following the Knudson two-hit tumorigenesis model whereby genetic predisposition (one mutated allele) is not enough to develop malignancy and somatic mutations in the target tissue are required to inactivate the remaining normal allele (6, 7).

## Pathology

Lung pathology in LAM is characterized by areas of thin-walled cysts co-existing with infiltrates of abnormal smooth muscle cells localized in the lung parenchyma, airways, lymphatics and blood vessels. Two subpopulations of smooth muscle or LAM cells have been identified: small spindle-shaped cells and larger epitheloid cells. In contrast to normal smooth muscle cells, LAM cells tend to proliferate in nodules with spindle-like cells placed in a center surrounded by epitheloid cells. Both cell types characteristically express smooth muscle cell markers, such as smooth muscle $\alpha$-actin, vimentin and desmin, as well as glycoprotein 100, commonly found in melanoma cells and immature melanocytes. LAM cells stain positively for human melanoma black (HMB45) antibodies, which recognize glycoprotein 100 and serve as diagnostic tools in LAM. Spindle cell proliferation of LAM cells is responsible for lung tissue damage, likely due to excessive matrix metalloproteinase (MMP) production (8). LAM cells are also present in lymphatic fluid-filled cysts (lymphangioleiomyomas) and renal angiomyolipomas. Smooth muscle cells from angiomyolipomas, as well as blood vessels and fat cells, are clonal, suggesting a common precursor (3).

## Pathogenesis

### *LAM cell proliferation: mTOR signaling*

As mentioned earlier, the development of sporadic and TSC-associated LAM has been linked to mutations in the tumor suppressor gene *TSC2,* resulting in abnormal mTOR-mediated signaling responsible for aberrant cell growth and proliferation. TSC1/TSC2 complexes negatively regulate mTOR signaling via inhibition of the phosphorylation of the ribosomal kinase S6K1 and the eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP1), the chief downstream targets of mTOR, which upon phosphorylation initiate cell processes that culminate in protein translation and subsequent cell growth (9). Activation of the mTOR signaling pathway, correlating with loss of *TSC2* (but not *TSC1*) has been found in sporadic angiomyolipomas (10). Analysis of *TSC2*-mutated LAM smooth muscle cells from lung isolates of LAM patients has evidenced constitutive activation of p70S6K (or S6K1), leading to abnormal hyperphosphorylation of the ribosomal protein S6 (11). Furthermore, activation of the p70S6K/S6 pathway correlated with elevated basal proliferative rates in primary cell cultures from patient-derived LAM cells (12). Restoring *TSC2* expression in LAM cells attenuated S6 hyperphosphorylation and p70S6K activity, as did the mTOR inhibitor rapamycin (sirolimus), which in addition to modulating p70S6K activity also inhibited abnormally high DNA synthesis. Thus, under physiological conditions, *TSC2* negatively regulates p70S6K activity and S6 phosphorylation, hence preventing protein translation and cell cycle progression. In LAM, however, loss of *TSC2* function would activate p70S6K/S6 signaling, resulting in benign tumor formation. Targeting this pathway with mTOR inhibitors, such as rapamycin, appears to be a major therapeutic strategy and is currently being tested in clinical studies (see Future trends).

### *LAM cell migration: the Rho signaling pathway*

LAM cells have been shown to spread through the lymphatics following a neoplasm-like paradigm (13, 14). *In vitro* studies have demonstrated that loss of *TSC2* function promotes migration and invasiveness of LAM cells via enhanced activation of the small GTP-binding protein Rho, which plays an important role in actin cytoskeleton regulation (15). In these studies, blocking Rho or its downstream signaling kinase ROCK (Rho-associated protein kinase) abolished LAM cell invasiveness. Further investigations revealed a role for TSC1/TSC2 complexes in the regulation of cell dynamics. As TSC1 possesses a Rho-activating domain which overlaps with the TSC2-binding site, it appears that in the

absence of a functional TSC2 (*i.e.,* LAM), TSC1 binds and activates Rho, causing the formation of actin stress fibers and focal adhesions, promoting cell motility and hence contributing to LAM pathology (16).

*Role of lymphangiogenesis in LAM progression*

The development of new lymphatic vessels, or lymphangiogenesis, has been implicated in LAM progression and severity. Histopathological studies of pulmonary and extrapulmonary LAM lesions have shown clusters of LAM cells wrapped by a monolayer of lymphatic endothelial cells. These round-shaped structures are able to disseminate through the lymphatic circulation and reach target tissues, in which LAM cells are liberated from the lymphatic endothelial lining and exposed to the extracellular matrix to cause new lesions via increased MMP secretion (14). Moreover, a correlation between LAM-associated lymphangiogenesis and the severity of pulmonary lesions has also been pointed out (17). Some studies have demonstrated the presence of key molecular mediators of lymphangiogenesis in LAM pathological lesions. Potent lymphangiogenic growth factors such as vascular endothelial growth factor (VEGF)-C and -D are expressed in LAM cells (17), while VEGF receptor 3 (VEGFR3) staining has been detected in lymphatic endothelial cells surrounding LAM cell clusters from pulmonary samples (14). The finding that VEGF-D levels are increased in the serum of LAM patients, together with LAM cell VEGF-D expression (18), suggests a potential role of LAM cells as the source of lymphangiogenic factors that would contribute to lymphangiogenesis, lymphatic endothelial cell spreading and subsequent LAM progression.

## Treatment

Current treatment options for LAM are aimed at delaying disease progression and alleviating the clinical symptoms (Table I). However, no effective treatment exists that is able to reverse pulmonary dysfunction.

*Hormonal therapy*

The fact that LAM occurs almost exclusively in premenopausal women and that pregnancy or exogenous estrogen exacerbate disease symptoms, suggested a hormonal dependence in disease pathogenesis. Moreover, estrogen receptor $\alpha$ (ER$\alpha$) expression has been detected in pulmonary LAM and angiomyolipoma cells. Progesterone has been commonly used for treating LAM, although with relative success. While some reports had indicated progesterone's potential benefits, retrospective analysis of LAM cases highlighted that progesterone treatment had no effect in delaying the progression of lung disease (19). In this study, patients treated with or without progesterone presented comparable rates of decline in lung function parameters, namely forced expiratory volume in one second ($FEV_1$) and diffusion capacity of the lung for carbon monoxide (DLCO), thereby discouraging the use of hormonal therapy in LAM. Although tamoxifen has also been used in some LAM cases, caution should be exercised as it has been reported to stimulate angiomyolipoma cell growth *in vitro* (20). Oophorectomy and gonadotropin-releasing hormone (GnRH) agonists, which were once used, are no longer recommended due to a lack of therapeutic benefit and the risks associated with permanent estrogen suppression (3). LAM patients should discontinue treatment with estrogen-containing medications and be advised of potential risks associated with pregnancy.

*Management of lung dysfunction*

Pulmonary LAM cell invasion and progressive cyst development invariably cause airflow obstruction and pneumothorax occurrence. Pneumothorax in LAM may arise from rupture of pleural cysts or by alveolar wall disruption, allowing air to invade the pleural cavity and causing lungs to collapse. More than 65% of LAM patients experience spontaneous pneumothorax as the primary symptom leading to diagnosis and are prone to recurrence, with an average of 3.5 episodes of pneumothorax per patient (1). The preferred intervention is pleurodesis or pleural symphysis, which induces generalized adhesion of pleural layers by means of chemicals, such as talc, or by surgery involving thoracotomy (mechanical pleurodesis). Almoosa *et al.* found both procedures equally effective for treating the initial pneumothorax event and preventing its recurrence in LAM patients (21). The severity of airflow obstruction, together with a decreased diffusion capacity of the lung for carbon monoxide, may often lead to respiratory failure requiring lung transplantation. A study supported lung transplantation as a valid treatment option in end-stage LAM patients, as the reported 1-year survival rate was 85.75%, which was superior to that of patients undergoing transplantation for other pulmonary conditions (22).

Bronchodilator treatment has also been reported to be beneficial in LAM patients, with response rates of around 25% (23). However, severely obstructed patients appear less likely to respond to acute bronchodilator therapy, as reported by Yen *et al.* (24). In general, smoking cessation is strongly recommended in LAM as it may aggravate pulmonary dysfunction.

*Treating angiomyolipomas*

The presence of renal angiomyolipomas usually does not compromise kidney function in LAM patients. However, if tumors exceed 4 cm they can cause hemorrhage which can in turn cause symptoms such as chronic flank pain and acute abdomen with hypovolemic shock. Hemorrhage may be treated with embolization or blood vessel cauterization. For large renal masses, surgical resection via nephron-sparing surgery may be the treatment of choice (1, 3).

*Table I: Summary of experimental therapies in lymphangioleiomyomatosis (from Prous Science Integrity®).*

| Drug | Design | Treatments | n | Conclusions | Ref. |
|---|---|---|---|---|---|
| Progesterone | Retrospective | Progesterone, p.o. or i.m. | 348 | No benefit was found for progesterone in decreasing the rate of decline in lung function | 19 |
| Sirolimus | Case report | Sirolimus, 4 mg/d x 8 mo | 1 | Sirolimus improved patient's general status, eliminated chylothorax and abdominal lymphangioleiomyomas, but had no effect on the number and size of pulmonary cysts | 25 |
| | Open | Sirolimus x 24 mo | 3 | Angiomyolipoma burden dramatically decreased after sirolimus treatment in patients with LAM and TSC. Lung function improved in 1 patient, whereas lung cyst pathology was not affected by sirolimus therapy | 26 |
| | Open | Sirolimus x 1 y | 20 | Sirolimus reduced angiomyolipoma burden in patients with TSC and sporadic LAM after 1 year. Sirolimus improved spirometric parameters and gas trapping in patients with LAM | 27 |
| | Open | Sirolimus | 35 | This trial will investigate the safety and efficacy of sirolimus in treating patients with angiomyolipoma of the kidney secondary to TSC or LAM | 28 |
| | Randomized Double-blind | Sirolimus, 2 mg p.o. x 1 y Placebo | 240 | The safety and efficacy of sirolimus in reducing LAM pulmonary manifestations will be assessed in this phase III trial (the MILES study) | 29 |
| Everolimus | Open | Everolimus x 2 y | 30 | The efficacy and safety of everolimus in reducing angiomyolipoma burden will be evaluated in patients with TSC or sporadic LAM | 30 |
| Doxycycline | Case report | Doxycycline, 20 mg/d →100 mg/d | 1 | Doxycycline treatment improved lung capacity of a 66-year-old woman diagnosed with LAM, from an $FEV_1$ value of 0.48 l before to 0.91 l after treatment. Monitoring of urinary MMP decrease was used as a measure of treatment efficacy | 36 |

TSC: tuberous sclerosis complex; LAM: lymphangioleiomyomatosis.

## Future trends in LAM therapy

### *Targeting the TSC2/mTOR pathway*

Sirolimus, also known as rapamycin, is an inhibitor of mTOR that has been used as an immunosuppressive agent for the oral prophylaxis of organ rejection since 1999, when it was launched by Wyeth Pharmaceuticals. Sirolimus efficacy has been reported in an isolated case of a 34-year-old woman with sporadic LAM featuring pulmonary cysts and chylothorax in the right lung, as well as abdominal lymphangioleiomyomas (25). After 8 months of treatment with sirolimus, chylothorax disappeared and pulmonary function greatly improved. Moreover, abdominal masses were also completely eliminated. However, sirolimus failed to reduce the number or size of lung cysts. Similarly, a marked decrease in angiomyolipoma burden was observed in 2 LAM and 1 TSC patient after sirolimus treatment (26). Results from a 1-year open-label trial showed that, in patients with TSC and LAM, sirolimus reduced the average total angiomyolipomata volume by around 50% and improved pulmonary function parameters after 1 year of therapy (27).

Currently, the Dana-Farber Cancer Institute, in collaboration with the National Cancer Institute (NCI), is investigating the safety and efficacy of sirolimus for the treatment of patients with kidney angiomyolipoma secondary to TSC or LAM (28). The Office of Rare Diseases has also initiated a larger randomized, double-blind, placebo-controlled study of sirolimus for the treatment of LAM, known as the MILES (Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus) study. Primary outcomes include lung function improvement, as

assessed by $FEV_1$ response, and the occurrence of adverse events after 1 year of treatment. The trial will also assess the efficacy of sirolimus in reducing other LAM pulmonary manifestations, such as chylothorax or lung cyst size (29).

Everolimus (Certican®; Novartis), another mTOR inhibitor structurally related to sirolimus, is also being studied in a small open-label trial in patients exhibiting angiomyolipomas due to TSC and sporadic LAM (30).

*Inhibition of extracellular matrix remodeling*

The destruction of lung parenchyma in LAM has been attributed to an increased expression and activity of MMPs, which create an environment prone to proteolysis. Several studies pointed out that signaling pathways involved in extracellular matrix (ECM) remodeling may also be important for LAM cystic pathology.

Early reports showed prominent immunoreactivity of MMP-2 and MMP-9 (and also MMP-1), which degrade collagen and elastin, in lung LAM cells compared to normal bronchiolar and vascular smooth muscle cells (31). Moreover, discoidin domain receptors (DDR) 1 and 2 —collagen receptors with tyrosine kinase activity— have been found to be upregulated in smooth muscle cells of LAM patients and their overexpression was found to reduce collagen production and enhance MMP activity (MMP-2 and -1), and subsequent collagen degradation (32).

Additionally, Zhe and colleagues described elevated serum response factor (SRF) levels in lung smooth muscle cells from LAM patients (33). SRF is a widely expressed transcription factor normally expressed in immature smooth muscle cells, which regulates gene expression associated with muscle differentiation. In LAM, abnormally high levels of SRF are thought to contribute to tissue damage via the induction of MMP expression (particularly MMP-2 and -14), as well as the downregulation of tissue inhibitor of metalloproteinase (TIMP)-3 (34). Moreover, SRF overexpression may also deregulate the plasminogen system by promoting its conversion to plasmin, which can degrade ECM directly or by activating MMPs (33).

Another factor implicated in ECM remodeling in LAM is transforming growth factor-β1 (TGF-β1), which has been detected in abundance in pulmonary LAM cell-rich foci, co-localized with fibronectin, a TGF-β1-induced molecule (35).

Thus, MMP inhibitors appear as potential drug therapies for the management of LAM. Moses *et al.* reported the successful use of doxycycline, a well-known tetracycline with MMP-inhibiting activity, in a LAM patient (36). After 6 months of doxycycline treatment, pulmonary function improvement was so remarkable that the patient abandoned the waiting list for lung transplant. In addition, urinary levels of MMP-9 and MMP-2 decreased after doxycycline exposure and were used as a biomarker of therapeutic efficacy. This approach has been claimed in the patent literature (37).

*Targeting the IFN/JAK/STAT pathway*

A role for interferon gamma (IFN-γ)/Janus kinase 1 (JAK1)/signal transducer and activator of transcription (STAT) signaling in LAM pathogenesis has been suggested by several authors. *In vitro* tuberous sclerosis models have shown that deletion of either *TSC1* or *TSC2* significantly suppresses IFN-γ expression, but increases STAT1 expression and STAT3 phosphorylation, and that exogenous IFN-γ treatment inhibits proliferation of *TSC1*- or *TSC2*-defective cells (38). Moreover, sirolimus treatment of these cells induces IFN-γ secretion. Similar findings were obtained in cells from sporadic LAM and renal angiomyolipoma cases that featured an absolute lack of IFN-γ expression and elevated STAT1 and STAT3 levels (39). Interestingly, TSC patients with *TSC2* mutations and presenting a high-expressing IFN-γ allele have shown a lower frequency of kidney angiomyolipomas (40). The use of IFN-γ in the management of angiomyolipomas has been claimed in the patent literature (41). Together, these findings encourage further clinical research of IFN-γ as a potential LAM therapy, alone or in combination with sirolimus.

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