Management of lymphangioleiomyomatosis
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Abstract

Lymphangioleiomyomatosis (LAM), a multisystem disease affecting almost exclusively women, is characterized by cystic lung destruction and presents with dyspnea, recurrent pneumothoraxes, chylous effusions, lymphangioleiomyomas, and angiomyolipomas. It is caused by the proliferation of a cancer-like LAM cell that possesses a mutation in either the tuberous sclerosis complex (TSC)1 or TSC2 genes. This article reviews current therapies and new potential treatments that are currently undergoing investigation. The major development in the treatment of LAM is the discovery of two mammalian target of rapamycin (mTOR) inhibitors, sirolimus and everolimus, as effective drugs. However, inhibition of mTOR increases autophagy, which may lead to enhanced LAM cell survival. Use of autophagy inhibitors, for example, hydroxychloroquine, in combination with sirolimus is now the subject of an ongoing drug trial (SAIL trial). Another consequence of mTOR inhibition by sirolimus is an increase in Rho activity, resulting in reduced programmed cell death. From these data, the concept evolved that a combination of sirolimus with disruption of Rho activity with statins (e.g. simvastatin) may increase TSC-null cell death and reduce LAM cell survival. A combined trial of sirolimus with simvastatin is under investigation (SOS trial). Since LAM occurs primarily in women and TSC-null cell survival and tumor growth is promoted by estrogens, the inhibition of aromatase to block estrogen synthesis is currently undergoing study (TRAIL trial). Other targets, for example, estrogen receptors, mitogen-activated protein kinase inhibitors, vascular endothelial growth factor-D signaling pathway, and Src kinase, are also being studied in experimental model systems. As in the case of cancer, combination therapy may become the treatment of choice for LAM.

Introduction

In this review we discuss the treatment of LAM, a multisystem orphan disease affecting almost exclusively women, which is associated with cystic lung destruction and extra-pulmonary abnormalities consisting of abdominal tumors (e.g. angiomyolipomas), lymphatic tumors (e.g. lymphangioleiomyomas), and chylous effusions (Table 1 and Figure 1) [1–4]. The pathological features of LAM result from proliferation of a neoplastic LAM cell that has characteristics both of smooth muscle cells and melanocytes [3]. Lung lesions consist of infiltrates of LAM cells in the walls of cysts and along blood vessels, lymphatics and bronchioles, leading to airway obstruction, vascular wall thickening, lymphatic damage, and venous occlusion [2,3]. LAM lesions comprise two types of cells: spindle-shaped and epithelioid [2,3]. Both cell types react with antibodies against smooth muscle antigens, for example, α-actin, vimentin and desmin. The epithelioid cells react with human melanin black antibody (HMB-45), a monoclonal antibody that recognizes a premelanosomal protein (gp100) that is encoded by the Pmel17 gene [2,3]. In the proper clinical setting, positive reaction to HMB-45 is virtually diagnostic of LAM [2–4].

LAM presents with dyspnea, recurrent pneumothoraxes, pleural effusions, ascites, and bleeding angiomyolipomas [4,5]. In most women, dyspnea and recurrent pneumothoraxes dominate the clinical picture, being a major cause of morbidity. In some cases, lung disease
progresses slowly, with decline in lung function leading to respiratory failure [5,6]. In others, usually younger women, LAM tends to run a more rapid course. Lung function abnormalities consist of decreased expiratory flow expressed as a reduction in forced expiratory volume in the first second (FEV₁), and decreased lung diffusion capacity (DLCO), leading to a reduction in breathing capacity and hypoxemia during exercise or at rest [1,5–7].

Two forms of LAM have been described. The inherited form of LAM is reported to occur in up to 81% of women with tuberous sclerosis complex (TSC) [8], an autosomal dominant disorder characterized by hamartomatous

| Pulmonary disease                  | Extrapulmonary disease               |
|------------------------------------|--------------------------------------|
| Thin-walled lung cysts             | Angiomyolipomas                      |
| Chylos pleural effusions           | Chylos ascites                       |
| Pneumothorax                       | Lymphadenopathy                      |
|                                    | Lymphangioleiomyomas                 |
|                                    | Meningiomas                          |
|                                    | Decreased bone mineral density       |

Figure 1. Computed tomography scan images of pulmonary and extrapulmonary features of lymphangioleiomyomatosis

Panel A: Computed tomography (CT) scan of the thorax showing numerous thin-walled cysts scattered throughout the lungs, which have almost completely replaced the normal lung parenchyma. Panel B: CT scan of the thorax showing a left chylothorax (black star). Panel C: CT scan of the abdomen shows a large angiomyolipoma in a patient with tuberous sclerosis complex and lymphangioleiomyomatosis (LAM). The fatty, low density component is clearly visualized and is indicated by the white star. Panel D: CT scan of the abdomen shows a large, fluid-filled lymphangioleiomyoma (white star) surrounding vascular structures.

Abbreviations: CT, Computed tomography; LAM, lymphangioleiomyomatosis.
tumors involving the central nervous system, skin, liver, heart and eyes, and associated with mental retardation, seizures and autism [9]. The sporadic form of LAM has been reported to occur in 3.3–7.7 million women [10]. In either form, LAM is caused by mutations in the tuberous sclerosis complex 1 (TSC1) or tuberous sclerosis complex 2 (TSC2) genes [11–13] that encode two proteins, hamartin and tuberin.

LAM is considered to be a low-grade malignancy. Data are consistent with a metastatic model. Identical TSC2 mutations have been found in the lungs and kidneys of the same patient with sporadic LAM [11,12]. Loss of heterozygosity of TSC2 has been demonstrated in LAM cells isolated from lung, angiomyolipomas, blood, chyle, and urine from patients with sporadic LAM and TSC-LAM [11–15].

LAM cells have been detected in donor lungs of patients who had lung transplantation [16,17]. These findings support the possibility that migration to the lungs of cells from other sites, such as the kidney, lymphatic system, or uterus may occur [16–18].

Hamartin and tuberin together inhibit the mammalian target of rapamycin (mTOR) signaling pathway, a major regulator of cell size and proliferation [19]. mTOR inhibitors, sirolimus and everolimus, have been proven effective in stabilizing lung function and reducing the size of chylous effusions, lymphangioleiomyomas and angiomyolipomas [20–22].

The severity of lung disease and its rate of progression is best assessed by clinical symptoms, histological grading of lung biopsy tissue, pulmonary function tests, computed tomography imaging, six-minute walk tests and cardiopulmonary exercise tests [5]. The clinical data are used to determine the need to treat patients with mTOR inhibitors.

In this article, we will focus on targeted therapies shown to be effective in LAM, and other agents that appear to be promising and are currently undergoing either preclinical or clinical testing. We will discuss how these potential treatments are expected to complement the actions of mTOR inhibitors. Finally, we will discuss the treatment of pneumothoraxes, chylous effusions, and angiomyolipomas as well as issues related to pregnancy and lung transplantation.

**General principles of management**

Patients should be told that LAM is a chronic disease with a median transplant-free survival time of approximately 29 years from the onset of symptoms and a 10-year transplant-free survival of 86% and that, as much as possible, they should lead a normal life [5,23,24]. Patients should be encouraged to lose excess weight, engage in physical activities, and exercise regularly. Levels of exercise should be limited only by the severity of lung disease. Sports involving physical contact and martial arts should be avoided because of the potential for bleeding in patients who have angiomyolipomas. Patients should be allowed to travel by land or air, except to high-altitude locations, depending on disease severity and risk of pneumothorax. The risk of a life-threatening pneumothorax associated with air travel is minor [25]. However, sudden onset of breathlessness or chest pain suggesting the presence of a pneumothorax should be investigated and ruled out prior to air travel. Arterial blood gases assist in determining whether a patient may travel by air without supplemental oxygen. A six-minute walk test or a cardiopulmonary exercise test to uncover exercise-induced hypoxemia and determine the need for supplemental oxygen is recommended [5,7]. Because of the potential risks of estrogens in the pathogenesis of LAM, a disease found primarily in women, patients should be advised against using estrogen-containing contraceptives and foods.

**Targeted therapies**

**Mammalian target of rapamycin (mTOR) inhibitors**

TSC1 and TSC2 encode, respectively, hamartin and tuberin [19,26–28], two proteins that regulate the intracellular serine/threonine kinase mTOR signaling pathway, a major regulator of cell size, proliferation and survival [19,26] (see Figure 2). mTOR is the catalytic subunit of two distinct complexes named mTOR complex 1 (mTORC1) and mTOR complex 2 (mTORC2) [19]. The hamartin/tuberin complex regulates mTORC1 negatively through its actions on Rheb (Ras homolog enriched in brain). Tuberin is a GTPase-activating protein for the guanine nucleotide-binding protein Rheb that converts active Rheb-GTP into the inactive Rheb-GDP form [19]. In the absence of functional tuberin, caused by TSC2 gene mutations, there is accumulation of active Rheb-GTP and stimulation of mTORC1. Activation of mTORC1 leads to phosphorylation of S6 kinase and eukaryotic initiation factor 4E-binding protein as well as increased protein translation, and cell size and proliferation [19,29]. mTORC2 is a mediator of actin cytoskeletal organization, cell cycle progression and cell survival [19,27], mTORC2 controls Rho and protein kinase B (Akt), and through its effects on Akt, promotes cell survival [19,27].

Sirolimus and everolimus are two immunosuppressant compounds that form a complex with FK506-binding protein-12, and inhibit mTORC1 [19]. mTOR inhibitors have been shown to decrease tumor size in the Eker rat
Tuberous sclerosis complex (TSC)1/2 integrates multiple signals to control cell size and proliferation. TSC1/2 regulates mammalian target of rapamycin (mTOR) complex 1 mTORC1 negatively through its actions on Rheb. Activation of mTORC1 leads to protein translation, cell growth and proliferation. mTORC1 is a major regulator of autophagy. Blockade of mTORC1 by sirolimus augments autophagy, leading to increased cell survival. This effect can be inhibited by hydroxychloroquine. mTOR complex 2 (mTORC2) regulates the actin cytoskeleton through Rho GTPases, which affect cell migration, morphogenesis and apoptosis. Simvastatin reduces Rheb and Rho activities and promotes apoptosis. Combined therapy with sirolimus, hydroxychloroquine or simvastatin may act synergistically to inhibit lymphangioleiomyomatosis (LAM) cell growth and promote apoptosis.

Abbreviations: Akt, protein kinase B; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; Rac, small GTPase binding protein of the Rho family; Rheb, Ras homolog enriched in brain; S6K1, S6 kinase 1; 4E-BP1, factor 4E binding protein 1; TSC, tuberous sclerosis complex.
model of TSC [30], decrease the growth of renal cystadenomas and liver hemangiomas in Tsc2 +/- mice, and decrease tumor growth and mortality in a mouse model with Tsc2 +/- tumors [31]. Several clinical studies have demonstrated the efficacy of mTORC1 inhibitors in the treatment of LAM [20–22]. A double-blinded, placebo-controlled study (MILES trial) testing the effect of sirolimus on pulmonary function was undertaken in 89 women with LAM. Forty-six patients were treated with sirolimus and 43 with placebo for 12 months [20]. Patients were followed for a year after discontinuation of therapy [20]. Compared to the placebo group, the sirolimus group had improvements from baseline in vital capacity, FEV_1, quality of life, and functional performance. After discontinuation of sirolimus, lung function decline resumed and was no different from that of the placebo group [20]. In another study [21], sirolimus was proven effective in reducing the size of chylous effusions, ascites, and abdominal lymphangioleiomyomas in 12 patients with lymphatic involvement who were treated for approximately 2.5 years (Figure 3). Nine of the 12 patients experienced complete resolution of their chylous effusions and abdominal lymphangioleiomyomas [21] (see Figure 3).

Based on current evidence, it is recommended that sirolimus or everolimus be used to treat LAM patients in whom lung function is declining rapidly or those who

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**Figure 3. Effect of sirolimus in lymphangioleiomyomatosis patients with chylous effusions and lymphangioleiomyomas**

Panel A: Changes in percent-predicted forced expiratory volume in the first second (FEV₁) before and during treatment with sirolimus in nine patients with lymphangioleiomyomatosis (LAM). During treatment, FEV₁ either increased or stabilized. Panel B: Changes in lung function during treatment with sirolimus in one patient with chylous pleural effusions and ascites. It can be seen that total lung capacity (TLC), forced vital capacity (FVC), FEV₁, and lung diffusion capacity (DLCO), increased during sirolimus therapy and that the effect was sustained beyond five years. Panels C and D: computed tomography (CT) scans of the abdomen show a large lymphangioleiomyoma before (white star) and after treatment with sirolimus. Complete resolution of the tumor was observed with sirolimus. Abbreviations: CT, computed tomography; DLCO, lung diffusion capacity; FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; TLC, total lung capacity.
have symptomatic lymphangioleiomyomas, chylous pleural effusions or ascites [20,21,32]. The role of sirolimus in patients with normal or stable lung function, or very slow rates of decline, is unclear. The starting dose of sirolimus should be 1 mg per day. Sirolimus serum levels must be monitored and dosage adjusted to attain serum trough levels between 5 and 15 nanograms per ml [20–22]. The most frequent adverse events associated with sirolimus therapy include stomatitis, hypercholesterolemia, upper respiratory tract infections, diarrhea, peripheral edema, acne, hypertension, headaches, leukopenia, delayed wound healing, thrombocytopenia, and proteinuria [20]. Close patient monitoring is necessary [20]. Laboratory tests including blood cells count, chemistries, urinalysis, urine protein/creatinine ratio, and sirolimus blood levels should be performed. Pulmonary function studies should be performed at least every six months. Interaction between sirolimus and other drugs and some foods, such as grapefruit, must be monitored carefully and adjustments to the dose of sirolimus made when appropriate. Currently, it is not known whether treatment must be continued for life or whether resistance to sirolimus eventually develops. The optimal dose of sirolimus that should be employed and when it is most efficacious to initiate treatment is not known. Dosage and blood levels currently used are based on experience with sirolimus in the prevention of graft rejection in patients who have undergone organ transplantation. It is possible that lower sirolimus levels may be equally effective, especially in the treatment of lymphatic disease [33].

**Statins**

Activation of mTORC1 and mTORC2, and increased Rho activity, are necessary for TSC2-dependent cell proliferation and survival [34]. Absence of TSC2 causes tuberin deficiency that results in increased Rheb and RhoA activity and enhanced cell survival [34]. In TSC2-deficient, rat-derived, TSC2-null ELT3 cells, down-regulation of RhoA increases apoptosis, suggesting that inhibition of RhoA, which is regulated by mTORC2, may reduce cell survival [34]. Since sirolimus and everolimus only suppress mTORC1, there is a rationale for therapies targeting mTORC2 signaling [34–36].

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme-A (HMG-CoA) reductase inhibitors that inhibit geranylerfanylation of Rho GTPases, and farnesylation of the small GTPases Ras and Rheb [37]. Atorvastatin was found to inhibit the growth of Tsc2<sup>−/−</sup> uterine-derived leiomyoma (ELT-3) and mouse embryonic fibroblasts by reducing Rheb activity [38]. Simvastatin, another HMG-CoA reductase inhibitor, was shown to inhibit RhoA activity (see Figure 4) and the proliferation of TSC-null cells and TSC2-null tumor growth in mice, and to promote apoptosis [34]. Combined treatment with sirolimus and simvastatin prevented recurrence of the tumors even after discontinuation of both drugs [34]. This effect was specific for simvastatin; atorvastatin did not reduce the size of liver and renal tumors in a mouse model of TSC [39]. Simvastatin was also shown to reduce alveolar space enlargement in a mouse model of LAM [40]. Further, combined with sirolimus, simvastatin blocked matrix metalloproteinase up-regulation and prevented alveolar destruction [40].

There are no data regarding the potential efficacy of simvastatin in the treatment of LAM. In one study, no correlation between statin use and angiomyolipoma response to sirolimus in patients with TSC or sporadic LAM was demonstrated [22]. In a retrospective study, the rate of decline in lung diffusion capacity in LAM patients treated with statins for hypercholesterolemia was greater than that of their matched, off-statin controls [41]. However, in this study, the number of patients treated with simvastatin was small. The effect of simvastatin combined with sirolimus or everolimus in the treatment of LAM is being investigated (NCT02061397).

**Anti-estrogen therapy**

Because LAM is predominantly a disease of pre-menopausal women, estrogens have been, from early on, implicated in its pathogenesis [4–6]. Oophorectomy, progesterone and gonadotrophin-releasing hormone (GnRH) analogues have all been used to treat LAM [6,42–46]. A number of case reports and uncontrolled studies have suggested beneficial effects of anti-estrogen therapies [42]. Others found no benefit from oophorectomy or progesterone therapy [43]. A reduced rate of decline in lung function in pre-menopausal patients treated with progesterone was reported [44], but a large retrospective study involving 275 patients reported no difference in disease progression between patients treated or not treated with progesterone [6]. Studies that tested the effect of GnRH analogues have also been inconclusive [45–46].

In vitro and experimental animal studies have provided a rationale for anti-estrogen therapy in LAM [47–50] (see Figure 4). LAM cells express estrogen receptors [51,52], and estradiol increases the proliferation of Eker rat-derived Tsc2-null, uterine ELT3 leiomyoma cells and the growth of xenograph subcutaneous tumors in vivo [48,49]. Further, estradiol also increases the number of circulating tumor cells, the survival of injected ELT3 cells, and the number of pulmonary metastasis of TSC-null cells injected subcutaneously in oophorectomized mice [49]. This effect of estradiol is associated with the activation of a mitogen-activated protein kinase (MAPK) signaling pathway that
Figure 4. Scheme depicting some potential new targeted therapies for LAM

Anti-estrogens

Estradiol

ERα

Transcription

MAPK pathway

MEK inhibitors

Inhibitors

VEGFR3
VEGF-C
VEGF-D

LAM cell survival and proliferation

MMP inhibitors

MMP Cathepsin

Lung tissue destruction

These include anti-estrogens, such as the aromatase inhibitor letrozole, ERα blockers, MAPK kinase inhibitors, MMP inhibitors, and inhibitors of VEGF receptors and growth factors. Abbreviations: ERα, estrogen receptor α; ERK, extracellular signal-regulated kinases; MAPK, mitogen-activated protein kinase; MEK, MAPK/ERK; MMP, metalloproteinase; VEGF-C, vascular endothelial growth factor C; VEGF-D, vascular endothelial growth factor D; VEGFR3, vascular endothelial growth factor receptor 3.
can be targeted in disease [35,36,49,53,54]. Activation of MAPK increases LAM cell resistance to apoptosis [35]. Estrogens also increase the proliferation of human TSC-heterozygous and TSC-null cells, and these effects are prevented by MAPK/ERK (MEK) kinase inhibitors [49] (see Figure 4). Finally, estradiol increases matrix metalloproteinase (MMP)-2 activity in LAM cells [50]. In a xenograph tumor mouse model where estradiol induces lung metastasis of TSC-deficient cells, Faslodex, the estrogen receptor antagonist, was shown to increase animal survival by inhibiting the formation of lung metastasis [55]. Faslodex also inhibited the estrogen-induced increase in MMP-2 and extracellular matrix remodeling [55].

Clinical studies aimed at either suppressing estrogen secretion with aromatase inhibitors or with estrogen receptor antagonists are warranted, using these drugs in combination with mTOR inhibitors (see Figure 4). A clinical trial (NCT01353209) examining the effect of the aromatase inhibitor letrozole in post-menopausal women, in whom the main source of estrogens are the adrenal glands, is currently under way.

Matrix metalloproteinases inhibitors

MMPs are proteases that degrade extracellular matrix and collagen and, by disrupting basement membranes, facilitate invasion by tumor cells [56–60]. LAM cells express immunoreactivity to MMP-2, MMP-9, and tissue inhibitors of metalloproteinase (TIMP), TIMP1, TIMP2 and TIMP3 [50,56–60]. Doxycycline, an MMP inhibitor, affects MMP production by TSC-null ELT3 cells [60,61] and inhibits MMP-2 secretion by TSC-null mouse embryonic and human LAM cells [62]. A potential role of doxycycline in the treatment of LAM has been suggested by several clinical observations [63,64]. Doxycycline was reported to decrease serum and urine levels of MMP-9 and MMP-2 and improve FEV₁ in LAM patients with mild disease [63]. A two year randomized placebo-controlled study just completed in 21 patients (NCT00989742) failed to show a significant difference in rates of FEV₁ decline between doxycycline and control groups, although the urinary levels of MMP-9 were decreased in the doxycycline group [65]. Currently there is no evidence that doxycycline is an effective treatment for LAM.

Inhibitors of autophagy

Autophagy is a mechanism by which cells maintain their energy homeostasis by degrading and recycling organelles, cellular debris, and damaged proteins [66–68]. These products are sequestered into autophagosomes which, after fusing with lysosomes, are degraded. The mTOR signaling pathway is a major regulator of autophagy [67–69]. Under nutrient-rich conditions, mTORC1 promotes cell growth and autophagy by inhibiting the human homolog of ATG1 (Unc-51-like kinase 1 [ULK1]) kinase complex, comprising ULK1, autophagy-related protein (Atg)13 and Atg17 [66,68]. Inhibition of mTORC1 increases autophagy and abrogates its inhibitory effects on phosphatidylinositol 3-kinase (PI3K) and Akt [69–71], which in turn leads to activation of mTORC1 [71,72]. Altogether, these actions lead to increased cell survival [71]. Sirolimus and everolimus, two mTORC1 inhibitors, stimulate autophagy by causing phosphorylation of Atg13, which blocks the inhibitory effects of ULK1 in the formation of autophagosomes [69–71]. Because LAM cells have low levels of autophagy, blockade of mTOR signaling with sirolimus may lead to increased LAM cell survival [69,71]. The antimalarial drug hydroxychloroquine blocks autophagy, inhibits the growth of cancer cells, and induces cell death [73] (see Figure 2). Sirolimus and hydroxychloroquine combined were found to be more effective than either drug alone in inhibiting the survival of TSC2-null cells and the growth of TSC2-null xenograph tumors, as well as in suppressing the spontaneous development of renal tumors in Tsc2<sup>+/−</sup> mice [69,72]. These observations provided a rationale for an on-going study (NCT01687179) testing the effect of hydroxychloroquine and sirolimus in LAM patients.

It has been proposed that other pharmacological agents inhibit the autophagy-promoting effect of mTORC1 inhibition by sirolimus and everolimus. A recent study showed that a combination of sirolimus and resveratrol, a polyphenol present in fruits and red wine, reduced sirolimus-induced autophagy and prevented Akt activation and apoptosis of TSC2-deficient cells [74]. Sirolimus and resveratrol in combination restored inhibition of Akt while blocking mTORC1 signaling, down-regulating autophagy and inducing apoptosis [74]. A reduction in autophagy was also shown to increase levels of Src kinase in TSC<sup>−/−</sup> cells and lung tissue of LAM patients [75]. Inhibition of Src reduced the invasiveness of TSC<sup>−/−</sup> cells and their colonization in the lungs [69], thus inhibiting the metastatic capacity of LAM cells. An Src inhibition trial is currently under way (NCT02116712).

These data indicate that inhibition of mTORC1 may affect LAM cell survival and its metastatic properties. The most effective treatment for LAM may evolve into a multidrug regime consisting of an mTOR antagonist along with pharmacologic agents that abrogate the undesirable effects of mTORC1 blockade. Human studies testing the effect of these compounds in combination with mTORC1 inhibitors may provide answers to these questions.
Potential future therapies

Several potential therapies for LAM are currently undergoing clinical trials. In addition, *in vitro* or animal data have suggested a potential beneficial role of other agents in the treatment of LAM [76–79].

Vascular endothelial growth factor receptor (VEGFR)-3 is a major regulator of lymphangiogenesis [54,80,81]. Immunoreactivity for vascular endothelial growth factors (VEGFs)-C and -D is present in LAM lesions and LAM cells [80]. In addition, because of the important role of lymphangiogenesis in the pathogenesis of LAM [54], and evidence that increased VEGF-D levels in the serum of LAM patients correlate with disease severity and clinical course [82], blockade of VEGFRs or anti-VEGF-D therapies are being considered as potential treatments [54,80,81]. In accordance, in a subcutaneous Tsc2−/− tumor mouse model, sorafenib (a Raf kinase and VEGF receptor pathway inhibitor) and sirolimus together decreased tumor volume and increased survival more effectively than sirolimus alone [76]. A member of the collagen IV family, named lamstatin [83], was recently reported to have anti-lymphangiogenic properties. Levels of lamstatin were found to be reduced in the lungs of LAM patients [83]. Further, *in vitro* and *in vivo* studies showed that lamstatin, combined with a 17-aminoacid peptide (CP17), inhibited proliferation and migration of human lung lymphatic endothelial cells, and decreased dysplasia of the tumor-associated lymphatic network in a lung adenocarcinoma xenograph mouse model [83].

Alterations in the interferon gamma (IFN-γ) signaling pathway were suggested by the finding that expression of IFN-γ is not present in angiomyolipoma from patients with TSC or LAM [77]. Treatment of Tsc1- or Tsc2-null cells with IFN-γ was shown to induce apoptosis [78]. There was evidence of synergism between IFN-γ and sirolimus in inducing apoptosis of these cells [78]. However, sirolimus and IFN-γ together were not more effective than sirolimus alone against TSC-related kidney tumors in Tsc2−/− mice [76]. A combination of an mTORC1 receptor antagonist and IFN-γ was more effective than either single agent in decreasing the severity of kidney cystadenomas and liver hemangio- mas in both Tsc−/− and Tsc−/− mouse models [31]. Both drugs together were also shown to be more effective than a single agent in reducing tumor growth in a mouse model of TSC [79]. Although IFN-γ appears not to directly inhibit the proliferation of Tsc2-null cells, or increase the inhibitory effects of sirolimus, there are data suggesting that increased expression of signal transducer and activator of transcription (STAT) 3 is present in LAM lungs and may possibly be a target for new therapies [84].

Finally, increased AMP-activated protein kinase (AMPK) activity has been reported in TSC tumors and Tsc-null cells and this may contribute to increased LAM cell survival [85]. Since Rheb controls AMPK activity independent of mTORC1 signaling [86,87] and Rheb depletion decreases tumorigenesis [87], targeting Rheb may have a role in combination therapy of LAM or TSC [87].

Treatment of complications

Pneumothorax

Once a patient has had a pneumothorax, the chances of having a recurrence are greater than 70% [1,88–90]. A small pneumothorax may be treated by chest tube drainage. If the air leak persists or the pneumothorax recurs, pleurodesis is recommended. A chemical or surgical pleurectomy by video-assisted thoracoscopic should be considered [88,90]. Chemical sclerosis with doxycycline, pleurectomy, mechanical abrasion and talc poudrage are also effective. Talc pleurodesis may result in considerable pleural scarring that may be associated with chronic pain as well as bleeding complications during removal of the native lungs at the time of transplantation [88,90].

Of great concern to patients with LAM is the risk of pneumothorax associated with air travel [25,91]. Since cabins of commercial aircraft are pressurized only to a barometric pressure equivalent to an altitude of 1,500 to 2,500 meters, during the airplane’s ascent or descent the cabin pressure falls or increases according to Boyle’s law [91], leading to gas expansion or contraction within body cavities, such as lung cysts or non-functioning bullae [91]. Retrospective studies indicate that the risk of developing a pneumothorax during air travel is small, ranging from 2–4 % [25,91,92]. There is no evidence that pre-existing chronic loculated pneumothoraces are associated with an increased risk of additional pneumothorax or expansion of a pre-existing pneumothorax during air travel [25]. Patients should be advised to take direct flights and to abstain from air travel if they are experiencing chest pain and/or increased dyspnea, until the presence of a pneumothorax is ruled out [25,92,93].

Another potential risk associated with flying is hypoxemia [91]. An arterial blood gas obtained while breathing room air may assist in deciding whether the patient may fly without supplemental oxygen. More precisely, arterial blood gases may be measured while they breathe a low oxygen concentration that produces an inspired oxygen pressure similar to that present in pressurized cabins of commercial airplanes.

Chylous effusions and lymphangioleiomyomas

Chylous pleural effusions, ascites, and lymphangioleiomyomas may compromise respiratory function and
cause abdominal pain, urinary frequency, obstruction, and peripheral edema [5]. Not uncommonly, patients may be misdiagnosed with a lymphoma or ovarian cancer [94–97]. The symptomatology associated with lymphatic disease in patients with LAM may cause a great deal of distress and poses difficult therapeutic problems. The least recommended therapeutic approach is continuous tube drainage of chyloous pleural effusions and ascites, as this approach results in nutritional compromise, protein loss, lymphopenia, increased risk of infections, and weight loss [21,88,90,98]. Further, once a thoracostomy tube is placed in the pleural cavity, drainage may become continuous and pleural symphysis may become difficult. It is preferable to perform therapeutic thoracentesis when patients become symptomatic. Low fat diet, pleuro-peritoneal or peritoneal-venous shunts, treatment with somatostatin and octreotide have been employed but there is little experience with these therapies in LAM [99–102].

Surgical pleurodesis may be effective in reducing the size of the effusion, but this will not ameliorate abdominal symptoms associated with ascites and lymphangioleiomyomas. To be effective, surgical pleurodesis must be performed under conditions of reduced chyle flow. To accomplish this, patients must be placed on parenteral nutrition with a fat-free solution before, during, and after pleurodesis. The thoracostomy tube should be removed only when daily drainage volume decreases to 200 ml or less.

A major advance in the treatment of lymphatic disease in LAM was the discovery that the mTOR inhibitor sirolimus decreased the volume of chyloous effusions, the size of lymphangioleiomyomas, and ascites, cleared lung infiltrates caused by lung lymph accumulation and dramatically improved lung function [21,103]. Consequently, sirolimus is now considered to be the treatment of choice (see Figure 3). Although volume reduction or complete resolution of the lymphangioleiomyomas generally occurs within a few months, resolution of chyloous effusions may take many months to more than a year [21]. While on sirolimus, therapeutic thoracentesis may be performed periodically if patients experience significant respiratory symptoms. Abdominal lymphangioleiomyomas must not be surgically removed or partially resected as this approach may result in ascites and disabling pleural effusions.

**Angiomyolipomas**

Angiomyolipomas occur primarily in the kidney and liver [104]. The major risk of angiomyolipomas is bleeding. Arterial embolization is recommended for the treatment of acute bleeding, severe pain, or prophylactically in patients with angiomyolipomas larger than 3–4 cm in diameter [104,105]. Resection of the kidney should be avoided unless the angiomyolipoma has an atypical radiologic appearance that raises the question of malignancy. In this case, a biopsy should be performed or the tumor should be resected [104,106,107]. mTORC1 inhibitors are effective in decreasing the size of renal angiomyolipomas in patients with TSC or sporadic LAM, with tumor size being reduced in about 44 to 50% of patients [22,108–110]. In one study, 42% of 79 patients with angiomyolipomas treated with everolimus responded with a 50% reduction in tumor size after 24 weeks of therapy [110]. Following discontinuation of the drugs, the angioliomas tended to return to their initial size. Treatment with mTOR inhibitors should probably be the initial approach for the treatment of large angiomyolipomas. Arterial embolization should be reserved for acute bleeding or for patients who do not tolerate mTOR inhibitors [104–110]. Once mTOR inhibitor therapy has begun, it must be continued because its discontinuation appears to return the tumor to its original size.

**Management of pregnancy**

By the time a young woman is diagnosed with LAM she may already have experienced one or more pregnancies. Not infrequently, a pneumothorax or increased dyspnea may uncover the presence of LAM [1,111,112]. Despite these events, of 353 pregnancies recorded in the LAM registry, 67% resulted in live birth and only about 17% had spontaneous abortion [1]. Of those who had been pregnant, 22% experienced worsening of respiratory symptoms [1]. Patients who were diagnosed with LAM during pregnancy appear to have had more premature births, and higher frequency of dyspnea and pneumothorax than patients diagnosed either before or after pregnancy [112]. These data, along with reports of worsening symptoms during pregnancy, such as dyspnea, pneumothorax, chyloous effusions or hemorrhage from angiomyolipomas [111,113–115], have raised the question as to whether LAM patients should be advised not to become pregnant.

Pregnancy should be discouraged in patients with moderate to severe disease or those in whom lung function is rapidly declining. The rationale for this recommendation is that these patients should be treated with sirolimus rather than risking further deterioration in lung function and a delay in starting therapy, because sirolimus must be discontinued prior to, and during pregnancy. Patients with mild disease who strongly desire to become pregnant should be told about its potential risks (e.g. pneumothorax, decline in lung function). They should be advised that with close medical and obstetrical supervision, others have tolerated pregnancy and
delivered a normal child. However, at present, there is no method of predicting the outcome in terms of frequency of complications such as pneumothorax or chylothorax, and the magnitude of potential decline in lung function.

**Lung transplantation**

Except in advanced stages, dyspnea at rest is not a major feature of LAM [5]. Patients with an FEV1 of less than one liter and a DLCO less than 30% predicted, who are receiving supplemental oxygen, might be comfortable at rest. However, exercise and hypoxemia requiring supplemental oxygen are major factors affecting the quality of life, namely the ability of patients to conduct activities of daily living. In one study [116], preoperative FEV1 and DLCO prior to transplantation were, respectively, 20±8 and 23±9% predicted and there was also resting hypoxemia. The average 6-minute walk test distance was 250 meters. The five-year post-transplant survival of LAM patients undergoing lung transplantation was around 69% [116–119]. The European experience with lung transplantation in LAM is similar to that of the USA [120], but others have reported five-year survivals of around 75% [121].

Because patients with very low FEV1 and DLCO on supplemental oxygen may live for many years [23,24], lung function needs to be severely compromised before lung transplantation is considered. We suggest that lung transplantation be discussed with the patient when FEV1 and DLCO are about 30% predicted, the patient is on continuous supplemental oxygen, is unable to carry out activities of daily living and has resting pulmonary hypertension. Importantly, the patient should rate her quality of life as being so poor that she wishes to undergo the additional risks associated with lung transplantation.

**Summary statement and conclusions**

LAM is a disease affecting women, which is associated with cystic lung destruction, and extrapulmonary manifestations consisting of abdominal angiomyolipomas and lymphangioleiomyomas. LAM presents with dyspnea, recurrent pneumothoraces, and hemorrhages from angiomyolipomas. LAM is characterized by a reduction in breathing capacity, hypoxemia during exercise or at rest, and respiratory failure.

Not long ago, LAM was defined as a fatal disease of young women for which there was no effective therapy and the only treatment option was lung transplantation. The establishment of the National Heart, Lung, and Blood Institute (NHLBI) LAM registry and other registries worldwide have led to major progress in the characterization of the clinical features and natural history of LAM. The finding that LAM is caused by mutations of TSC1 or TSC2 genes that encode hamartin and tuberin, two proteins with a major role in control of the mTOR signaling pathway, led to therapies targeting mTOR. Two mTORC1 inhibitors, sirolimus and everolimus, have been shown to be effective in stabilizing lung function, and reducing the size of chylous effusions, lymphangioleiomyomas, and angiomyolipomas.

Treatment with these pharmacological agents, which is generally tolerated by patients, is now the standard therapy for patients showing compromised lung function or those who have symptomatic chylous effusions and lymphangioleiomyomas or large angiomyolipomas.

In the case of angiomyolipomas, discontinuation of mTORC1 inhibitor therapy results in a return to pretherapy tumor size, suggesting that to sustain the therapeutic effects of mTORC1 inhibitors, treatment has to be continued. mTORC1 inhibition results in increased autophagy and possibly enhanced LAM cell survival, thus reducing the beneficial effects of sirolimus or everolimus. Inhibition of autophagy with hydroxychloroquine has been suggested as a new treatment for LAM, complementing inhibition of mTORC1 with sirolimus or everolimus. Such a therapeutic regimen is now undergoing clinical testing.

Deficiency of tuberin due to TSC2 mutations, as occurs in LAM, results in increased RhoA GTPase activity and increased cell survival. This effect is mediated through mTORC2 signaling. Since sirolimus and everolimus only affect the activity of mTORC1, there is a rationale for therapies targeting RhoA GTPases. Statins inhibit Rho GTPases and promote apoptosis. Simvastatin combined with sirolimus is effective in preventing lung destruction in a mouse model of LAM. Simvastatin combined with sirolimus or everolimus is currently undergoing phase 1 clinical testing in patients with LAM.

Other treatments that are being investigated are estrogen receptor blockers, aromatase inhibitors, MAPK/ERK (MEK) inhibitors and VEGFR antagonists. Blockade of VEGF receptors or anti-VEGF therapies may be of value in the treatments of LAM in view of the role of lymphangiogenesis in its pathogenesis and evidence that increased levels of VEGF-D in the serum of LAM patients correlates with disease severity and clinical course. Preclinical studies have shown that a VEGF pathway inhibitor and sirolimus together decreased tumor volume in an animal model of LAM and increased survival more effectively than sirolimus alone.

We conclude that, as in the case of cancer, LAM (which is considered a low grade malignancy) may be best treated...
with multiple drugs targeting signaling pathways considered important in the pathogenesis of LAM.

Abbreviations
Akt, protein kinase B; AMPK, AMP-activated protein kinase; Arg, autophagy-related protein; DLCO, lung diffusion capacity; FEV1, forced expiratory volume in the first second; GnRH, gonadotrophin-releasing hormone; HMB, human melanin black antibody; IFN-γ, interferon gamma; LAM, lymphangioleiomyomatosis; MAPK, mitogen-activated protein kinase; MMP, metalloproteinase; mTOR, mammalian target of rapamycin; mTORC1, mTOR complex 1; mTORC2, mTOR complex 2; PI3K, phosphoinositide-3-kinase; RhoB, Ras homolog enriched in brain GTPase-activating protein; TSC, tuberous sclerosis complex; ULK1, Unc-51-like kinase 1; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Disclosures
The authors declare that they have no disclosures.

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