Published in final edited form as:

Expert Opin Orphan Drugs. 2016 ; 4(4): 369–378. doi:10.1517/21678707.2016.1148597.

EPIDEMIOLOGY, PATHOGENESIS and DIAGNOSIS of LYMPHANGIOLEIOMYOMATOSIS

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Abstract

Introduction—Lymphangioleiomyomatosis (LAM) is a disease of women characterized by cystic lung destruction, lymphatic involvement, and renal angiomyolipomas.

Areas covered—LAM is caused by proliferation of abnormal smooth muscle-like LAM cells containing mutations and perhaps epigenetic modifications of the *TSC1* or *TSC2* genes, which encode, respectively, hamartin and tuberin, two proteins controlling the mechanistic target of rapamycin (mTOR) signaling pathway. LAM occurs sporadically or in association with tuberous sclerosis complex. LAM may present with dyspnea, recurrent pneumothorax or chylothorax. Pulmonary function tests show reduced flow rates and lung diffusion capacity. Exercise testing may reveal hypoxemia and ventilatory limitation. The severity and progression of disease may be assessed by computer tomography, and pulmonary function and exercise testing. mTOR inhibitors, (e.g., sirolimus) are effective in stabilizing lung function, and reducing the size of chylous effusions, lymphangioleiomyomas, and angiomyolipomas.

Expert opinion—Different clinical phenotypes including variable rates of disease progression and variable responses to therapy are seen in LAM patients. No one test is available that predicts the course of disease at the time of diagnosis. Further research regarding the molecular biology of LAM clinical phenotypes is warranted. Recent advances in the characterization of the pathogenesis of LAM are leading to the development of new therapies.

Keywords

Lymphangioleiomyomatosis; Tuberous sclerosis complex (TSC); *TSC 1* and *TSC2* mutations; mTOR

1. Definition and Epidemiology

Lymphangioleiomyomatosis (LAM) is a multisystem disease that affects almost exclusively women, and is characterized by cystic lung lesions and extrapulmonary features consisting of renal angiomyolipomas and lymphatic involvement e.g., lymphangioleiomyomas, chylous

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Declaration of interest

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effusions [1-4]. The histopathologic features of LAM derive from the proliferation of phenotypically heterogeneous cells (LAM cells), which in the lung, have characteristics of smooth muscle cells and melanocytes, and contain mutations and perhaps epigenetic modifications of the tuberous sclerosis complex (TSC) *TSC1* or *TSC2* genes [1-4]. Two forms of LAM have been described. The sporadic form, which occurs in 3.3-7.7/million women [5], and the inherited form of LAM, which may occur in up to 80% of women with TSC [6]. TSC is an autosomal dominant disorder that is characterized by hamartomatous tumors involving the central nervous system, skin, liver, heart and eyes, and is associated with intellectual impairment, seizures and autism [7]. LAM may also occur in 13-38 % of men with TSC [7,8]. Sporadic LAM has been shown to be caused by mutations of the *TSC2* gene [9-11]. TSC is caused by mutations of either the *TSC1* or *TSC2* genes [8-11], with *TSC2* gene mutations usually resulting in a more severe clinical phenotype.

The *TSC1* and *TSC2* genes encode two proteins, hamartin and tuberin, that together inhibit the mechanistic target of rapamycin (mTOR) signaling pathway, a major regulator of cell size and proliferation [12].

Sporadic LAM is a chronic multisystem disease that can affect both pre- and post-menopausal women with an estimated median transplant-free survival of approximately 29 years from the onset of symptoms. The 10-year transplant-free survival has been reported at 86 % [13,14].

TSC is an autosomal-dominant disorder, which occurs in one of 12,000-14,000 children under the age of 10, or 1 in 6,000 live births [8]. TSC is characterized by facial angiofibroma, periungual fibromas, Shagreen patches, cortical tubers, cardiac rhabdomyomas, giant-cell astrocytomas, autism, intellectual impairment and seizures [8]. Although the prevalence of LAM in women with TSC had been estimated to be around 26 % [15], a more recent study reported that the prevalence of LAM in women increases with age and may be up to 80 % [6]. Men with TSC and lung cysts show less clinically significant disease than women [7].

2.Pathology

Lymphangiomyomatosis is classified as a low-grade metastasizing neoplasm by the World Health Organization classification of lung tumors. It has been placed in the category of PEComatous tumors, along with clear cell tumor or perivascular epithelioid cells tumors (PEComa) [16].

The pathological features of LAM consist of proliferation of atypical hyperplastic smooth muscle cells, around and along lymphatic channels, obstructing airways, veins and lymphatics, which may be associated with lymphadenopathy, involvement of the thoracic duct, angiomyolipomas, chylous effusions, and lung hemorrhage with hemosiderosis [17,18]. LAM lesions in the lungs are characterized by proliferation of neoplastic smooth muscle-like cells (LAM cells) in small clusters located on the edges of cysts and along blood vessels, lymphatics and bronchioles [3]. LAM cell infiltrates cause obstruction of the airways, vascular wall thickening, disruption of the lymphatic vessels, and venous occlusion

and hemorrhage [4,19]. Nodular lesions consist of spindle-shaped cells in the center, and epithelioid cells with large cytoplasm at the periphery [4]. Enlargement of the air spaces is associated with the proliferation of type II pneumocytes and destruction of elastic and collagen fibers in the walls of the cysts [4]. Both the spindle-shaped and the epithelioid LAM cells react with antibodies against smooth muscle antigens, e. g., α -actin, vimentin, desmin [4]. The epithelioid cells also react with human melanin black antibody (HMB-45), a mouse monoclonal antibody that recognizes gp100, a premelanosomal protein encoded by the *Pmel17* gene [20] (Figure 1 A). The reactivity of LAM cells to HMB-45 is localized to intracellular structures characteristic of melanosomes [20]. Positive immunoreactivity to HMB-45 has also been reported in angiomyolipoma smooth muscle cells, and clear cell tumors, e.g., PEComas. The spindle-shaped cells react with anti-proliferation cell nuclear antigen (PCNA) antibodies, indicating a proliferative phenotype [4,20]. In addition, LAM cells exhibit positive immunoreactivity for estrogen and progesterone receptors [20,21], β -catenin [22,23], E-catherin [24], and epidermal growth factor receptor [25]. The antipeptide antibody α PEP13h, which reacts with a C-peptide terminal of the Pmel17 protein, and identifies over 82 % of the LAM cells in lung nodules whereas HMB-45 identified only 25 % [26].

Angiomyolipomas are tumours that consist of aberrantly differentiated cells containing three dysmorphic elements resembling, adipocytes, smooth muscle-like cells and vascular cells [4]. AML occur as discrete individual tumors within the normal kidney or liver parenchyma that range in size from microscopic to more than 20 centimeters in diameter [4,27].

Lymphangioliomyomas are chyle-filled encapsulated lymphatic masses of varying sizes, which are most frequently located in the retroperitoneum, pelvis, and posterior mediastinum [27]. These tumors consist of LAM cells arranged in fascicular, trabecular and papillary patterns containing slit-like vascular channels [4]. Both spindle-shaped and epithelioid LAM cells are represented in these tumors and show reactivity to α -actin, myosin, and desmin [4]. Obstruction of chyle flow by proliferation of epithelioid LAM cells in the walls of lymphatic vessels appears to be responsible for formation of the chyle-filled lymphangioliomyomas.

3.Clinical Presentation

The two most frequent clinical presentations of LAM are recurrent pneumothoraces and dyspnea (Table 1) [1-3]. Less frequently, the first manifestation of LAM is a chylous effusion, abdominal or pelvic tumors, hemoptysis, abdominal hemorrhage caused by a renal angiomyolipoma or the incidental discovery of lung cysts and/or abdominal tumors [1-6]. Pneumothoraces may occur in about 50-60 % of patients with LAM and recurrence is common [1-3]. Patients with larger cysts are more likely to present with pneumothoraces [38]. LAM patients also frequently present with dyspnea on exertion, which tends to progress with time. These patients have more advanced disease at the time of presentation than those who present with pneumothorax, perhaps because the occurrence of a pneumothorax results in lung imaging studies that uncover the presence of cystic lung disease [13,14]. Investigators however, have proposed that patients who present with dyspnea on exertion represent a different subgroup than those who present with pneumothoraces [29].

Other modes of presentation include chylothorax, chylous ascites, hemoptysis, chyluria, chyloptysis, and posterior mediastinal, retroperitoneal and pelvic lymphangioleiomyomas suggesting malignancy, or hemorrhage from renal angiomyolipomas (Table 1) [1,4,27].

In patients with TSC, skin lesions such as Shagreen patches, facial angiofibromas, and periungual fibromas, and brain involvement including tubers and giant cell astrocytomas may be seen [8]. Women with TSC and LAM present with features distinct from those seen in patients with sporadic LAM. LAM-TSC patients tend to be diagnosed at an earlier age than sporadic LAM patients [30]. Further, the proportion of LAM-TSC patients with mild disease, normal lung function and low rates of functional decline is greater than in sporadic LAM [30]. However, this finding may reflect the fact that LAM-TSC may be more easily diagnosed in patients with less severe lung disease [30]. Despite these differences, young women with TSC and lung cysts warrant close medical follow-up because rapid deterioration in lung function may occur [30].

The prevalence of angiomyolipomas in LAM-TSC is higher than in sporadic LAM and most patients have bilateral AML [30,31]. In sporadic LAM the prevalence of AML is 50 % or less and AML are frequently unilateral [30,31]. In addition, AML are much more likely to grow and bleed in LAM-TSC than in sporadic LAM [32]. Sclerotic bone lesions are more frequent in patients with TSC-LAM [31]. Lymphatic involvement consisting of lymphangioleiomyomas and chylous effusions is more common in sporadic LAM than in LAM-TSC: 38 % versus 13 % [30].

4.Pathogenesis

4.1. LAM as a low-grade malignancy

The WHO classified LAM as a low-grade malignant neoplasm, being listed as a soft tissue neoplasm similar to PEComas, leiomyomas and sarcomas [16]. Evidence favoring the malignant nature of LAM cells comes from multiple sources. When lungs from male donors were transplanted to female LAM patients invasion by the recipient's LAM cells was demonstrated [33,34]. Consistent with this metastatic potential, LAM cells have been identified in blood, chylous effusions and urine of LAM patients [35-37], LAM cells with *TSC2* loss of heterozygosity (LOH) were demonstrated in body fluids of more than 90 % of the patients [37].

4.2. LAM cell markers

In addition to expressing markers of melanosomal cells, e.g. gp100, LAM cells also express estrogen and progesterone receptors [21], vascular endothelial growth factor receptor 3 (VEGF-D3) [38-44], chemokine receptors [45,46], CD44v6, a glycoprotein associated with metastasis [47], osteopontin, a regulator of CD44 [47], and matrix metalloproteinases (MMP) [48].

Vascular endothelial growth factor-D (VEGF-D) is a lymphangiogenic growth factor that is elevated in the serum of patients with LAM [38-44], more in patients with lymphatic disease than in those with AML or only cystic lung disease [38-41]. VEGF-D is of value as a diagnostic tool and as a marker of lymphatic involvement [38-44]. An association between

serum levels of VEGF-D and the severity of lung disease, as graded by use of oxygen and severity of airflow obstruction, and the rate of decline in lung function has been reported [38,40,42,43]. Measurement of VEGF-D in the serum is now an accepted method of establishing a diagnosis of LAM in patients who have no extrapulmonary disease or TSC, and to exclude patients with other cystic lung diseases [38,40,41]. Serum levels above 800 pg/ml in the setting of cystic lung disease are diagnostic of LAM [42].

Chemokines interact with receptors on the cell surface and participate in the homing of metastatic cells to specific organs [45,46]. Chemokine receptors most commonly found in lung tissue sections and bronchoalveolar lavage fluid cells, are CCR2, CCR7, CCR10, CXCR2, CXCR1, and CXCR4. CCL2/MCP-1 selectively attracts LAM cells and its production is regulated by tuberin. Immunoreactivity to CCL2/MCP-1 was identified in LAM nodules of 70% of 30 patients with LAM [45]. MCP-1, a chemokine that stimulates angiogenesis, fibrosis, and recruitment of monocytes, is overexpressed in angiofibroma and periungual cells from TSC patients and in *Tsc2*^{-/-} cells; this is caused by loss of tuberin function [46]. A subset of CCL2 gene polymorphisms was found to be more frequent in LAM patients than in healthy subjects and it correlated with the rate of decline in FEV₁ [45].

CD44 is a transmembrane glycoprotein adhesion molecule involved in cell migration and growth. It binds to metalloproteinases, osteopontin and hyaluronic acid to play an important role in cancer progression [47]. CD44v6, a CD44 variant generated as a result of alternative splicing, is found on the surface of LAM cells, in lung nodules [47]. Cells expressing CD44v6 have loss of TSC2 heterozygosity. CD44v6 may enhance the metastatic potential to the LAM cells by enabling them to adhere to the extracellular matrix. Serum levels of osteopontin, a regulator of CD44 gene expression and alternative splicing, are elevated in the serum of LAM patients [47].

4.3. Mechanism of LAM cell proliferation

LAM cell proliferation is thought to be caused by dysregulation of the mechanistic Target of Rapamycin (mTOR) signaling pathway. Hamartin and tuberin, proteins encoded respectively, by *TSC1* and *TSC2* genes (Figure 2), regulate the intracellular serine/threonine kinase mTOR signaling pathway, that controls cell size, proliferation and survival by integrating signals from growth factors, energy, and stress [49,50]. Tuberin is a regulator of cell cycle, cell growth and proliferation and hamartin is important in the organization of the actin cytoskeleton [49,50]. There are two main complexes involving mTOR: mTORC1, which includes raptor (regulatory-associated protein of mTOR) and is sensitive to mTOR inhibitors, e.g., sirolimus, everolimus [50], and mTORC2, which contains rictor (rapamycin-insensitive companion of mTOR) and is not sensitive to mTORC1 inhibitors sirolimus or everolimus (Figure 2) [51-53].

Tuberin, a GTPase-activating protein for the guanine nucleotide-binding protein Rheb (Ras homolog enriched in brain), promotes the formation of inactive Rheb-GDP from active Rheb-GTP [49,50]. Absence of tuberin, as occurs with *TSC2* gene mutations, loss of heterozygosity, or inhibition of the hamartin-tuberin complex by growth factors either through the mitogen-activated protein kinase (MAPK) or insulin-signalling pathways, leads to accumulation of active Rheb-GTP, stimulation of mTOR, phosphorylation of S6 kinase

and eukaryotic initiation factor 4E-binding protein, and increased translation, cell size and proliferation [54] (Figure 2).

4.4. Role of estrogens

A role of estrogens in the pathogenesis of LAM has long been suspected because of the fact that LAM occurs almost exclusively in women [1-3]. In addition, LAM lung nodules and angiomyolipoma have been shown to have estrogen and progesterone receptors [21,56,57]. Furthermore, LAM is frequently observed in pre-menopausal women [1-4], and worsening of pulmonary symptoms has been reported during pregnancy or subsequent to the administration of exogenous estrogens [58,59]. In addition, the rate of decline in lung function appears to be greater in pre-menopausal than in post-menopausal women [60].

In-vitro and animal data have also shown that estrogens promote the proliferation of TSC-null rat ELT3 leiomyoma-derived cells, stimulate the growth of human angiomyolipoma *TSC2*^{-/-} cells, and promote the survival of pulmonary metastasis of *Tsc*^{-/-} ELT3 cells in mice [61-63]. Estrogens have also been shown to stimulate activation of extracellular signal-regulated kinase 2 (ERK2) and the transcription of *Fra1*, a gene involved in the estrogen-stimulated invasive phenotype of LAM cell [64]. Finally, there is evidence that mTOR and estrogen-signaling pathways and metalloproteinases (MMPs) have an important role in promoting the migration and invasiveness of LAM cells [65].

4.5. Mechanism of cyst formation

The mechanisms by which LAM cell proliferation causes formation of lung cysts and destruction of the lung parenchyma is poorly understood [66]. One hypothesis is that the airways are compressed by proliferating LAM cells, and this causes distention of the terminal airspaces upstream from the occluded airways, leading to cyst formation [2,66]. Degradation of lung elastic fibers caused by proteinases would be another possible cause of the cystic lesions [66]. Matrix metalloproteinases (MMP) have an important role in remodeling, lymphangiogenesis, angiogenesis, cell migration, human cancer and metastasis [67]. LAM nodules contain MMP2, MMP9, MMP1, MMP activators (MT1-MMP) and inhibitors (TIMPs) [48,68]. Positive immunoreactivity of LAM cells with anti-MMP-2 antibodies and increased amounts of MMP-1, MMP-9, and MMP-13 in skin tumors of TSC patients have been reported [69,70]. Levels TIMP-3, were found to be reduced in LAM nodules [68], and serum levels of MMP-9 are higher in patients with LAM than in normal subjects [70]. These data suggest that an excessive production of matrix metalloproteinases may contribute to lung destruction [66]. Overexpression of MMP and cathepsin K could lead to degradation of collagen, elastic fibers, and lung matrix [72]. It has been suggested that the lung remodeling observed in LAM results in a VEGF-D and VEGF-C-mediated increased expression of MMP2, MMP9 and cathepsin-K [65,66,68].

4.6. Source of LAM cells

A likely source for LAM cells migrating to the lungs are renal angiomyolipomas [66]. However, less than half of the patients with sporadic LAM have these tumors. Other sources for LAM cells that have been proposed are uterine leiomyomas and perivascular epithelioid cell tumors (PEComas) which are prevalent in patients with LAM [73]. In patients with

lymphangioliomyomas and lymphadenopathy LAM cells originate from these lymphatic structures [74]. The identification of LAM cell clusters (LCC) comprising LAM cells surrounded by lymphatic endothelial cells in the lymphatic fluid of LAM patients would be another point of entry of LAM cells into the circulation [36,65]. These data suggest a role of abnormal lymphangiogenesis in the pathogenesis of LAM [36,65,74]. LAM nodules contain cleft-like spaces lined by endothelial lymphatic cells, which express VEGF3R [36,65,74]. From the lymphatic vessels, LCC would be able to reach the venous system and systemic circulation and, eventually lodge in the lung capillaries.

5. Physiologic Features

Air flow obstruction occurs in approximately 61 % of patients with sporadic LAM [1,30,60] (Table 2). Normal spirometry may be seen in about 31 % of patients with sporadic LAM, and in 53% of the patients with TSC-LAM (Table 2) [1,30,60]. Air trapping may be seen in patients with severe airflow obstruction. Lung diffusing capacity (DL_{CO}) is reduced in about 57 % of patients with sporadic LAM [1]; some patients with reduced DL_{CO} may have normal flow rates [30,60]. Exercise abnormalities consisting of reduced exercise capacity, decreased peak oxygen uptake, reduced breathing reserve, increased ventilatory equivalent for carbon dioxide, and hypoxemia, are frequently observed in LAM [75,76].

Airflow limitation in LAM is thought to be due predominantly to increased airway resistance and not caused by loss of lung elastic recoil [77,78]. The main causes of dyspnea and exercise limitation are reduced breathing reserve, dynamic hyperinflation, and an exaggerated ventilatory response to exercise because of impaired oxygen transfer due to loss of alveolar capillary surface area [75,76,79]. Exercise-induced pulmonary hypertension, which may be seen in patients with severe lung disease, may also be a factor limiting oxygen transfer [80].

6. Radiologic Features

Chest radiographic findings may be normal or show an interstitial pattern or cystic changes. Computed tomography (CT) shows well-defined, round, thin-walled cysts throughout the lungs; cysts may vary in size from a few millimeters to two centimeters (Figure 3 A,B) [81,82]. In patients with lymphatic involvement, pleural effusions and lung infiltrates due to accumulation of chyle in the interstitium may be present (Figure 3 B) [81-83]. CT indices of lung disease severity have been correlated with pulmonary function, and exercise performance [76,84,85,86,87].

Angiomyolipomas, lymphadenopathy, lymphangioliomyomas and ascites may be visualized by ultrasonography, CT scans or magnetic resonance imaging (Figure 3 C, D) [27]. Large AML may hemorrhage spontaneously (see Figure 4) [27]. Atypical angiomyolipomas lacking fatty tissue, comprise predominantly epithelioid LAM cells but may also represent renal cell carcinoma [88].

Lymphangioliomyomas appear as well-circumscribed masses of variable dimensions, comprising an outer wall and a fluid-rich region central region (Figure 3 C) [27,89]. The size of lymphangioliomyomas tends to be less in the morning or in the fasting state than in the

evening, after meals [90-92]. These changes in tumor size may help to differentiate lymphangioleiomyomas from malignant tumors [90-92].

7. Diagnosis

The diagnosis of LAM should be suspected in any young woman who presents with progressive dyspnea, recurrent pneumothoraces, or chylous pleural effusions [1-3]. Not infrequently these patients are diagnosed with asthma, physical unfitness or psychosomatic disorders. Pulmonary function testing is the simplest method to test for pulmonary disease. If the test shows evidence of air flow obstruction and/or impaired diffusion capacity, CT scan of the chest and abdomen should be performed to detect the presence of lung cysts and abdomino-pelvic abnormalities, namely AML or lymphangioleiomyomas.

A diagnosis of definite LAM can be made by a CT scan showing the characteristic lung cysts and a transbronchial, thorascopic or open lung biopsy [93-95], showing the histological features of lymphangioleiomyomatosis including immunoreactivity of LAM cells with monoclonal antibody HMB 45 [20]. The presence of a characteristic lung CT scan and AML, chylous effusions, lymphangioleiomyoma or TSC, are sufficient to establish the diagnosis of LAM [95]. The presence of a characteristic CT scan in the absence of extrapulmonary findings or TSC is not sufficient to make a diagnosis of definite LAM [95]. However, measurement of serum VEGF-D, a lymphangiogenic growth factor that is increased in the serum of patients with LAM, especially those with lymphatic involvement, may assist in the diagnosis [40,41]. In the presence of consistent clinical data and characteristic radiologic findings, serum levels of VEGF-D equal or greater than 800 pg/ml are now accepted as being diagnostic of LAM and extremely rare in other cystic lung diseases [40-42].

The differential diagnosis of LAM includes Langerhans cell histiocytosis, Birt-Hogg-Dubé syndrome, follicular bronchiolitis, light chain disease, Sjogren's syndrome, allergic alveolitis, bronchiectasis, Meunier-Kuhn syndrome, emphysema, sarcoidosis, pneumoconiosis and chronic lung infectious processes. In general, most of these conditions can be easily excluded on the basis of clinical, histopathological, and radiologic findings [2,95].

8. Prognosis

Clinical, pathologic, and physiologic data assist in assessing the prognosis of newly diagnosed patients with LAM. Young, pre-menopausal patients tend to have a faster decline in lung function than older, post-menopausal women [60,96]. Those patients with a past history of pneumothorax as the first manifestation of LAM tend to present with better lung function than those who seek medical help because of dyspnea on exertion [13,29]. Patients presenting with dyspnea appear to have worse prognosis than patients whose initial symptom is a pneumothorax [29]. In a study performed in Japan, the rate of functional decline in patients with FEV₁ and DL_{CO} above 40 % predicted, presenting with dyspnea, was greater than in those presenting with pneumothorax [29].

In patients who have had a lung biopsy, a predominance of cystic lesions instead of cellular infiltrates along with the presence of hemosiderin-laden macrophages in the biopsy tissue is associated with more severe disease and worse prognosis [19,97]. Patients with more cystic disease tend to have lower FEV₁, DL_{CO}, lower peak exercise oxygen uptake, greater exercise-induced hypoxemia, and reduced survival than patients with predominantly nodular lesions [19,76].

Pulmonary function testing is the simplest method of assessing the severity of lung disease in LAM [1,60]. However, in patients with near-normal flow rates and a reduced diffusion capacity, the severity of lung disease may also be graded by a 6-minute walk test or cardiopulmonary exercise testing [75,76,85]. Rates of FEV₁ and DL_{CO} decline over time help in defining whether lung disease is rapidly declining or slowly progressing [60,96]. A positive response to bronchodilators, which occurs in 25% to 30% of LAM patients, may also be a predictor of disease severity and rate of progression [97,98].

VEGF-D levels correlate with DL_{CO} and CT scan as measures of severity of lung disease [38,40]. Serum levels of VEGF-D correlated positively with use of oxygen, bronchodilator response, lower quality of life, decreased ability to perform daily living activities, lower diffusion capacity, and more severe airflow obstruction [52].

9. Expert opinion

In the last two decades there have been major advances in unraveling the natural history of LAM, and its pathogenesis, and pathophysiology. Instead of considering LAM a rare fatal disease that has no effective treatment and may require lung transplantation, physicians can now advise patients that LAM is a chronic disease spanning decades and that many patients live long lives. Furthermore, treatment, which is beneficial to many patients, is now available. Despite great progress, major gaps in our knowledge about the pathogenesis of LAM, and the factors determining the clinical phenotypes and rate of progression of lung disease are still present. It is unknown why some patients experience slow progression of disease to live long normal lives and eventually die from other more common diseases, and others have accelerated progression of disease advancing from an asymptomatic stage to lung transplantation in a matter of several years. Although in general, LAM may be more aggressive in younger women, it may also progress in post-menopausal older women and require pharmacologic therapy or lung transplantation. No test is available to predict the course of the disease at the time of diagnosis. Currently, one is limited in the monitoring of lung disease with pulmonary function studies or imaging studies. Another gap in our knowledge concerns the different clinical phenotypes presented by LAM patients. It appears that some patients presenting with recurrent pneumothoraces tend to have larger cysts with preservation of lung parenchyma in between the cystic lesions. These patients may have a “different” type of LAM than those who present with dyspnea, chylous effusions, hemoptysis or lymphangioleiomyomas. Indeed, the latter clinical presentation is seldom associated with pneumothoraces, and may be associated with chyloptysis, a normal FEV₁, a reduced DL_{CO} and silent exercise-induced hypoxemia. The response to therapy could potentially be different in these types of patients. Only by defining the molecular biology of these clinical phenotypes shall we be able to find specific and effective treatments for LAM.

Major advances in the characterization of the pathogenesis of LAM have been translated into development of new therapies. The finding that the mTOR signaling pathway has a major role in the regulation of LAM cell proliferation and growth lead to new effective treatment with mTOR inhibitors. Treatment with sirolimus or everolimus has been shown to stabilize or improve lung function and reduce the size of chylous effusions, lymphangioleiomyomas and angiomyolipomas. However, inhibition of mTORC1 results in increased autophagy and cell survival. New treatments, namely inhibitors of autophagy, e.g., hydroxychloroquine, and RhoA GTPases, e.g., simvastatin, are being tested. Other mechanisms, such as those involving the role of estrogens, lymphangiogenic growth factors, chemokines, and metalloproteinases in the pathogenesis of LAM, are potential targets for therapeutic interventions. In agreement, other treatments under investigation are, estrogen receptor blockers, aromatase inhibitors, blockade of VEGF receptors and anti-VEGF-D therapies are also under consideration.

Acknowledgments

This paper has been supported by the Intermural Research Program, National Heart Lung and Blood Institute of the NIH, grant number 95-H0186.

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Article highlights

- LAM is a multisystem disease characterized by cystic lung destruction, abdominal angiomyolipomas, and lymphatic disease. Two forms of LAM have been described, a sporadic form and a form associated with the Tuberous Sclerosis Complex (TSC).
- LAM is a low grade malignancy caused by mutations of the *TSC1* or *TSC2* genes, which encode respectively, hamartin and tuberin, two major regulators of the mTOR signalling pathway. Hamartin and tuberin deficiency causes proliferation and growth of abnormal smooth muscle-like cells (LAM cells).
- LAM patients frequently present with recurrent pneumothorax, progressive dyspnea, chylous effusions, and hemorrhage from renal angiomyolipomas.
- The clinical course of LAM is highly variable. The severity of disease at the time of diagnosis tends to be less in patients presenting with pneumothoraces than in patients presenting with dyspnea. LAM tends to progress more rapidly in younger, pre-menopausal patients. At the time of diagnosis, no one test can predict the prognosis of LAM. The molecular biology underneath the various clinical phenotypes of LAM is unknown.
- LAM may be diagnosed by imaging studies showing the typical round thin-walled cysts scattered throughout the lungs, in the presence of TSC or extrapulmonary involvement. Lung biopsy or serum levels of VEGF-D equal to or above 800 pg/ml along with characteristic clinical and computed tomography findings are considered to be diagnostic of LAM.
- LAM patients should have close medical follow-up, consisting of pulmonary function tests, exercise testing and imaging studies, to monitor disease progression.
- Major advances in the characterization of the pathogenesis of LAM have been translated into development of new therapies. Treatment with sirolimus or everolimus stabilize or improve lung function and reduce the size of chylous effusions, lymphangiomyomas and angiomyolipomas. Because inhibition of mTORC1 results in increased autophagy and cell survival, inhibitors of autophagy, and RhoA GTPases, are now being tested.
- Other mechanisms, such as those involving the role of estrogens, lymphangiogenic growth factors, chemokines, and metalloproteinases in the pathogenesis of LAM, are also potential targets for new therapies.

Figure 1. Pulmonary histopathological findings of LAM. Panel A: Lung LAM nodule showing the characteristic spindle and epithelioid LAM cells. The right side of the panel demonstrates HMB45 immunoreactivity (brown color) of LAM cells. Panel B: Histopathology of the lung showing cystic lesions and smooth muscle cell-like infiltrates (black arrows) on the walls of the cysts (black asterisks).

Figure 2.

Simplified scheme of the mTOR signaling pathways. TSC1/2 integrates multiple signals, such as those from growth factors, energy state and hypoxia, to control cell size and proliferation. TSC1/2 regulates mTORC1 negatively through its actions on Rheb. Activation of mTORC1 leads to cell growth and proliferation. mTORC2 regulates the actin cytoskeleton through Rho GTPases, which affect cell migration, morphogenesis and survival. Absence of TSC1/2, e.g., hamartin, tuberlin, results in loss of Rheb inhibition,

leading to activation of mTORC1, enhanced 4EBP1/S6K signaling and increased cell size and proliferation. Sirolimus blocks mTORC1, decreasing cell size and proliferation.

Abbreviations : mTOR: mechanistic target of rapamycin; mTORC1: mTOR complex 1; mTORC2: mTOR complex 2; Rheb GAP: Ras homolog enriched in brain GTPase-activating protein; S6K: S6 kinase 1; 4E-BP1: factor 4E-binding protein 1.

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Figure 3. Computed tomography scans of women with LAM showing pulmonary and extrapulmonary radiologic features of LAM. Panels A : Lung CT scan shows numerous cysts scattered throughout the lungs. Panel B: Lung CT scan of a LAM patient with lymphatic involvement. Thickening of the interlobar fissures and interstitial infiltrates indicate the presence of chylous fluid in the pleural space and lung parenchyma. Panel C: A large pelvic lymphangioliomyoma is seen adjacent to the right iliac artery. Panel D: Large left renal angiomyolipoma (white asterisks), intermixed with normal kidney tissue (black asterisk).

Figure 4.
Computed tomography scans image showing a large right renal angiomyolipoma complicated by severe bleeding. Large renal and perirenal hematomas (white asterisks) are seen surrounding the kidney parenchyma (black asterisk).

Table 1
Clinical manifestations of LAM *

	Sporadic LAM	TSC-LAM
Dyspnea	73 %	71 %
Pneumothorax	57 %	47 %
Abdominal angiomyolipomas	41 %	96 %
Chylous effusions	20 %	14 %
Lymphangioleiomyomas	38 %	13 %
Hemoptysis	10 %	3 %

*
Data from references 1 and 30

Table 2
Pulmonary function abnormalities in LAM *

Functional abnormality	Sporadic LAM	TSC-LAM
Decreased FEV ₁	61 %	38 %
Decreased FEV ₁ /FVC ratio	61 %	38 %
Decreased DL _{CO}	57 %	38 %
Increased RV/TLC ratio	7 %	3 %
Normal lung function	30 %	53 %

* Data from references 1 and 60. Abbreviations: FEV₁, forced expiratory volume in the first second; FVC, forced vital capacity; DL_{CO}, lung diffusion capacity for carbon monoxide; TLC, total lung capacity. RV, residual volume.