Recent advances in the pathobiology and clinical management of lymphangioleiomyomatosis

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Purpose of review
Lymphangioleiomyomatosis (LAM) is a rare systemic disease that occurs almost exclusively in women. In the last few years, our understanding of disease pathobiology has improved substantially; in addition, a guideline document has recently been developed that provides recommendations for the diagnosis and clinical management of patients with LAM. Yet, significant gaps in knowledge remain.

Recent findings
Groundbreaking insights into the cellular biochemistry of LAM have led to the reclassification of the disease as a low-grade, destructive, metastasizing neoplasm. In addition, recent data confirm the potential of next-generation sequencing to detect low-prevalence mutations in tuberous sclerosis (*TSC*) genes in sporadic LAM. A randomized, double-blind, multicentre trial has confirmed the efficacy of sirolimus in stabilizing lung function, improving functional performance and quality of life, and reducing lymphatic manifestations in patients with LAM. Accordingly, recent guidelines issued by the American Thoracic Society and the Japanese Respiratory Society recommend sirolimus treatment for patients with LAM and reduced lung function. Uncertainty remains, however, with regard to patient selection, and timing of initiation, duration and dosing of treatment.

Summary
Significant advances have been made in the diagnosis and clinical management of patients with LAM. However, additional studies are needed to assess long-term safety and efficacy of sirolimus therapy, and to identify predictors of disease behaviour and response to treatment.

Keywords
genetics, lymphangioleiomyomatosis, rapamycin, sirolimus, treatment, tuberous sclerosis complex

INTRODUCTION

Lymphangioleiomyomatosis (LAM) is a rare, low-grade, metastasizing neoplasm with almost exclusive female occurrence that arises from an unknown source, spreads via the lymphatics and targets primarily the lung, resulting in cystic remodelling [1]. The disease, which may occur either sporadically or in the context of tuberous sclerosis complex (TSC) – an autosomal dominant neurocutaneous disorder – is characterized by widespread proliferation of smooth-muscle-like cells (LAM cells) that harbour mammalian target of rapamycin (mTOR)-activating mutations in *TSC* genes leading to uncontrolled cellular growth, motility, and survival [2]. Disease pathogenesis remains poorly understood. However, production by the LAM cells of metalloproteases and lymphangiogenic growth factors, such as vascular endothelial growth factor (VEGF)-D, is believed to contribute to cystic destruction of the lung and development of lymphatic manifestations of the disease (i.e. chylous effusions and abdominal lymphangiomyomas), respectively [1].

In the last decade, growing scientific interest in LAM coupled with patient advocacy-driven collaborative efforts has produced remarkable insights

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KEY POINTS

- Major insights into the cellular and molecular mechanisms of LAM have led to the reclassification of the disease as a low-grade, metastasizing neoplasm.
- Evidence-based guidelines exist that provide recommendations for the diagnosis and clinical management of patients with LAM.
- Sirolimus stabilizes lung function, and reduces lymphatic manifestations in patients with LAM; however, sirolimus does not cure the disease and durable benefit requires prolonged treatment.
- Predictors of disease behavior and response to treatment will greatly facilitate clinical decision-making and conduction of clinical trials of novel therapies.

into molecular mechanisms of the disease and has led to the identification of an effective therapy (e.g. sirolimus) that is recommended by current guidelines [3▪▪]. In this review, we summarize and critically discuss the most relevant recent literature on LAM cell biology, genetics, and pharmacological treatment. We also highlight gaps in knowledge and provide hints for further research in this field.

LYMPHANGIOLEIOMYOMATOSIS: A NEOPLASTIC DISEASE

In the WHO classification of lung tumours, LAM is categorized as a perivascular epithelioid cell tumour (PEComa) [4]. Indeed, LAM lesions are characterized by the infiltration and proliferation of small clusters of LAM cells that accumulate to produce cystic destruction of the lung parenchyma and distortion of the lymphatic vessels. Two types of LAM cells are recognized: small, spindle-shaped cells that express smooth muscle proteins such as α-actin, vimentin, desmin, and proliferating cell nuclear antigen (PCNA); and large, epithelioid cells that display a high degree of immunoreactivity with human melanin black (HMB)-45 antibody and with antibodies against oestrogen and progesterone receptors [5]. LAM cells express also CD44v6, a glycoprotein that might contribute to the metastatic potential of LAM cells by conferring them the ability to adhere to the extracellular matrix [6,7]. Circulating LAM cells can be isolated in the blood, urine, and chylous effusion from LAM patients [8]. Moreover, LAM may recur in transplanted lungs, although this a relatively rare and often asymptomatic occurrence. In fact, recurrent LAM has been identified mainly in biopsies performed for other purposes, and recurrent LAM does not seem to affect survival [9–11]. LAM cells found within the allograft express *TSC* mutations from the host, consistent with a model of metastatic spread of the disease [12–14].

Matrix metalloproteinases (MMPs) detected within LAM lesions are believed to be responsible for the structural changes characteristic of the disease. Indeed, MMPs can destroy the extracellular matrix, thus promoting cell migration and cystic destruction of the lung [15]. According to this pathogenetic hypothesis, MMP-3 inhibitor is downregulated in LAM lesions [16], whereas serum levels of MMP-9 are significantly elevated in LAM patients compared with normal controls [17]. In addition, cells lacking *TSC1/TSC2* overexpress MMPs [18]. Another feature of pathogenetic relevance is the loss of heterozygosity (LOH) within *TSC* genes. Mutant *TSC* genes can modify the regulation of the mTOR signalling pathway, aberrantly activated in a number of human tumours [19▪]. LAM cells displaying *TSC2* LOH can be isolated from blood cultures and from other body fluids in patients with LAM [8,20], and treatment with sirolimus decreases detection of LOH [21]. Therefore, circulating LAM cells might potentially be exploited to confirm the diagnosis of LAM, and to identify patients not responding to sirolimus therapy [22]. Recently, Steagall *et al.* [23] observed different LOH patterns in the same patient; this genetic heterogeneity in circulating LAM cells, whether because of multiclonal origin or genetic instability over time, corroborates the hypothesis of LAM as a low-grade neoplasm.

GENETICS OF TUBEROUS SCLEROSIS COMPLEX AND LYMPHANGIOLEIOMYOMATOSIS: AN EVOLVING STORY

TSC is an autosomal dominant disorder caused by mutations in either the *TSC1* or *TSC2* gene, which cause loss-of-function of the TSC1 or TSC2 proteins, referred to as hamartin and tuberin, respectively [19▪]. Two-thirds of cases of TSC occur in patients without a family history, and are because of *de novo* germline heterozygous mutations. Notably, in about 10% of cases of TSC, no mutation in the *TSC1* or *TSC2* genes is identified; most of such cases are because of either genetic mosaicism of *TSC* genes or to an intronic mutation that could not be detected by conventional screening methods [19▪,24,25]. In contrast, sporadic LAM may be caused by either a somatic mutation (second hit) in addition to a germline mutation in *TSC1* or *TSC2* (first hit), or two, postembryonic somatic mutations in *TSC2* and/or LOH in *TSC2*. Fujita *et al.* [26] performed a deep next-generation sequencing analysis of *TSC1* and *TSC2* genes using genomic DNA

from blood leukocytes, LAM tissue from lung, LAM cultured cells, or LAM cell clusters from patients with sporadic LAM. As expected, no germline mutations within *TSC1* or *TSC2* were detected. Conversely, somatic mutations were identified in six of nine patients with mutant allele frequencies of 1.7–46.2%. Three of these six patients harboured two different *TSC2* mutations. Additional mutations of lower frequency were also found, consistent with the hypothesis that LAM tissues are likely to be composed by heterogeneous cell populations [26]. Phenotypic heterogeneity between sporadic LAM and TSC–LAM suggests that gaps in knowledge remain regarding possible additional unidentified genes, gene modifiers, and signalling networks involved.

DIFFERENCES AND SIMILARITIES BETWEEN SPORADIC LYMPHANGIOLEIOMYOMATOSIS AND TUBEROUS SCLEROSIS COMPLEX–LYMPHANGIOLEIOMYOMATOSIS

TSC is characterized by neurologic conditions, such as seizures, cognitive disability and autism, and benign tumours in multiple organs, including renal angiomyolipomas [27]. TSC is an autosomal dominant disease; yet, approximately two-thirds of patients do not have a family history of TSC, which explains why up to half of the cases are diagnosed in their adulthood [28]. The presence of angiomyolipomas increases the odds of a LAM patient having TSC–LAM, whereas the presence of lymphangioleiomyomas decreases the odds of a LAM patient having TSC–LAM [29]. Contrary to earlier series reporting a low prevalence of clinically significant LAM in patients with TSC [30], more recent series found a prevalence of LAM – defined as multiple lung cysts – ranging from 26 to 49% of TSC patients [31–36]. In the TuberOus SClerosis registry to increase disease Awareness (TOSCA), the largest clinical case series of TSC to date [37▪], only 6.9% had LAM diagnosed, confirming that LAM is underdiagnosed in TSC patients in clinical practice.

TSC–LAM is generally associated with milder symptoms than sporadic LAM. However, in a study of 79 women with TSC–LAM, two had lung transplants and two died of respiratory failure [28]. In addition, in a cohort of 284 patients with TSC followed for over three decades, 16 died from complications of TSC, including two who died from LAM [38]. Comparison of severity, however, may be confounded by recruitment bias, with TSC–LAM being more often detected as an incidental finding or as part of screening for cystic lung disease in adults with TSC [39]. In a large study of patients with LAM followed up over a period of 16 years, Taveira-DaSilva *et al.* [29] compared the volume occupied by lung cysts and the rate of decline in lung function between 40 patients with TSC-LAM and 40 patients with sporadic LAM matched for age and lung function. TSC-LAM patients were diagnosed at an earlier age and had more preserved lung function than patients with sporadic LAM, but yearly rates of change in forced expiratory volume in 1 s (FEV1), diffusing capacity of the lung for carbon monoxide (DL_{CO}) or cyst scores were comparable between the two conditions. Although some patients with TSC-LAM experienced rapid progression to severe lung disease (particularly women aged 18–30 years), the proportion of patients with abnormal lung function and higher rates of FEV1 decline was greater in sporadic LAM.

Overall, these data support the recommendation that women aged 18–30 years with TSC undergo radiological testing for lung cysts, and that those with TSC-LAM be followed with serial lung function to determine disease progression and potential need for therapy. The most reliable method of assessing the prognosis of LAM is to measure the annual rate of decline in lung function and the change in the number and volume of lung cysts over time, with a chest CT performed every 2–3 years.

TREATMENT WITH SIROLIMUS: UNANSWERED QUESTIONS

mTOR sirolimus is a standard therapy for LAM as it improves lung function (as measured by FEV1 and forced vital capacity (FVC)), functional performance and quality of life, and reduces lymphangioleiomyomas, chylous pleural effusion, and renal angiomyolipomas [3▪▪]. In addition, sirolimus reduces the number of circulating LAM cells [21,22]. Although current guidelines recommend sirolimus treatment for patients with LAM with abnormal lung function (defined as a FEV1 $<$70% predicted) uncertainties remain with regard to patient selection, and timing of initiation, duration and dosing of treatment. Everolimus seems to have comparable efficacy and tolerance as sirolimus, although these two drugs have not been formally compared [40].

Patient selection and timing of treatment initiation

In clinical trials of LAM, abnormal lung function has been defined as FEV1 less than 70% predicted, and the recommendations of the guidelines have been formulated accordingly [3▪▪]. Yet, patients with LAM may be symptomatic despite normal or near-normal

spirometry, and this may be because of an elevated residual volume (>120% predicted) or reduced DL_{CO} (<80% predicted). In a recent real world observational study, Bee *et al.* [41▪▪] examined lung function response and side effects to sirolimus in 47 women with LAM and progressive lung disease who had been treated with sirolimus for longer than 1 year. Overall, mean change in FEV1 over time (ΔFEV1) was +11 ml/year, although between individuals, ΔFEV1 varied from +254 to −148 ml/year. Notably, when patients were divided into quartiles according to their ΔFEV1, those with shorter disease duration and less severely impaired lung function pretreatment displayed a significantly better response to sirolimus. However, previous data from a prospective analysis of the Multicentre International LAM Efficacy of Sirolimus (MILES) trial showed that baseline serum VEGF-D levels correlated with disease severity – as defined by the need for supplemental oxygen, pulmonary function measures, and worsening symptoms – and that incremental increases in baseline VEGF-D concentrations were associated with incremental benefit from sirolimus treatment [42]. Interestingly, low VEGF-D concentrations (e.g. <0.8 ng/ml) were associated with slow disease progression in a minority of patients in the placebo group and poor response to treatment in the sirolimus group, suggesting that perhaps in patients with LAM and normal VEGF-D level disease pathogenesis may be less dependent on TSC2-regulated activation of mTORC1 [43]. In this regard, the development of a prediction model of response to treatment based on clinical, radiological, biomarker, and molecular data would greatly help identify patients more likely to benefit from sirolimus therapy. Moreover, some patients experience progressive decline in lung function despite treatment, the benefit of which may be only temporary [44], highlighting the need for further new treatment perspectives [45].

At present, it is unclear whether asymptomatic patients with normal or mildly impaired lung function should be treated. Clinical observation without drug therapy is generally recommended in these patients, mainly because of lack of supporting evidence, and concerns about the cost and long-term tolerance and safety of sirolimus, although some experts consider offering sirolimus therapy to patients with an annual FEV1 loss of at least 90 ml/year, which is at least threefold higher than the normal rate of FEV1 loss [3▪▪]. The currently ongoing Multicenter Interventional Lymphangioleiomyomatosis Early Disease (MILED) trial has been designed to determine whether early, long-term (2 years), low-dose (fixed at 1 mg/day) sirolimus treatment will prevent disease progression in patients with well preserved lung function (FEV1 >70% of the predicted value; NCT03150914).

Treatment duration

Current guidelines recommend sirolimus treatment for LAM patients based on its ability to stabilize lung function and to reduce the volume of chylous effusions and lymphangioleiomyomas [14]. Durable benefit requires prolonged – and potentially lifelong – treatment, but limited data exist on the risks and benefits of long-term sirolimus treatment in patients with LAM [14]. In one study, the beneficial effects of sirolimus was sustained over a period of approximately 5 years, with no clear evidence of resistance to the drug. Adverse events, although common, were generally mild and manageable [46]. The ongoing Multicenter International Durability and Safety of Sirolimus in LAM Trial (MIDAS), which is an observational 'registry' trial of patients with LAM who are on treatment or are being considered for treatment with mTOR inhibitors, will help refine treatment for LAM and determine whether long-term therapy with sirolimus or everolimus is well tolerated and efficacious (NCT02432560).

Treatment dosing

In the MILES trial, the dose of sirolimus was adjusted to maintain serum trough levels between 5 and 15 ng/ml [44]. However, a retrospective observational study of 15 patients with LAM treated for more than 6 months suggested that low-dose sirolimus (e.g. serum trough level ≤5 ng/ml) might be similarly effective in improving lung function and reducing chylous effusion [47]. The optimal dose of sirolimus is unknown; however, lower dose sirolimus might provide similar benefits with reduced drug-related side effects and potentially improved safety of long-term treatment.

PROGNOSTIC FACTORS OF AN 'UNPREDICTABLE' DISEASE

In LAM, little is known regarding individual factors that may predict severe decline in lung function and risk of progression to chronic respiratory failure, which might be used to guide treatment indications at an early stage of disease. The MILES trial showed that sirolimus stabilizes lung function in patients with FEV1 70% or less of the predicted value [44]. However, patients with FEV1 greater than 70% were excluded from the trial, and those with isolated reduction in DL_{CO} 70% or less were not enrolled either. A guideline statement from the European Respiratory Society and American Thoracic Society

Table 1. Diagnostic criteria for lymphangioleiomyomatosis[a]

Presence of TSC
Renal angiomyolipoma(s)
Elevated serum VEGF-D greater than 800 pg/ml
Chylous effusion (pleural or ascites) confirmed by tap and biochemical analysis of the fluid
lymphangioleiomyomas (lymphangiomyomas)
Demonstration of LAM cells or LAM cell clusters on cytological examination of effusions or lymph nodes
Histopathological confirmation of LAM by lung biopsy or biopsy of retroperitoneal or pelvic masses

[a]A definite diagnosis of LAM can be established if a patient with compatible clinical history and characteristic chest HRCT abnormalities has one or more of the features listed above. Data from [53▪,54]. HRCT, high-resolution computed tomography; LAM, lymphangioleiomyomatosis; TSC, tuberous sclerosis complex; VEGF-D, vascular endothelial growth factor-D.

(ATS)/Japanese Respiratory Society (JRS) suggests that sirolimus be considered for patients with rapid decline in lung function [3▪▪,48], although there is no definition for 'rapid' decline. Indeed, the mean rate of decline in lung function varies greatly between studies [49–51] as progression of disease may be affected by variables such as menopausal status, diagnosis of TSC-LAM versus sporadic LAM, or response to bronchodilators, among others. Nevertheless, it has been recently shown that premenopausal women whose FEV1 or DL_{CO} are declining faster than expected based on their individual rates of functional decline should be considered for sirolimus therapy before they cross the 'therapeutic' threshold of 70% predicted FEV1 [52▪].

Serum levels of VEGF-D may help establishing the diagnosis of LAM when elevated in a patient with compatible cystic lung disease. However, because serum VEGF-D is elevated (>800 pg/ml) in only about 70% of patients with LAM, normal VEGF-D levels do not rule out LAM [3▪▪] (Table 1). In the MILES trial, a higher baseline VEGF-D level was associated with better lung function response in the sirolimus group [42,44]. In addition, patients with a serum VEGF-D level greater than 800 pg/ml may experience a faster decline of FEV1 than those with lower levels [54]. However, whether VEGF-D can be used as a predictor of disease progression and of response to treatment needs to be explored further. Finally, serum VEGF-D levels may be greater among LAM patients with lymphatic involvement than in those without [55].

PHARMACOLOGICAL TREATMENT: BEYOND MAMMALIAN TARGET OF RAPAMYCIN INHIBITORS

The ATS/JRS guidelines recommend against the use of doxycycline (conditional recommendation based on low-quality evidence) as well as hormonal

Table 2. Overview of ongoing clinical trials of lymphangioleiomyomatosis

Therapeutic intervention	Primary outcome	Phase	Recruitment status	Clinical trial identifier
Resveratrol in combination with sirolimus vs. sirolimus	Change in serum VEGF-D level after 24 weeks of treatment	II (open label)	Recruiting	NCT03253913
Nintedanib as a single agent	Changes in FEV1 at week 52	II (open label)	Recruiting	NCT03062943
Saracatinib as a single agent	Changes in FEV1 over a 9-month period	II (open label)	Recruiting	NCT02737202
Albuterol	Greater improvement in FEV1 with nebulized vs. inhaled albuterol	II (open label)	Recruiting	NCT01799538
Sirolimus or everolimus	Safety and durability of mTOR inhibitors over at least 2 years	Longitudinal observational study	Recruiting	NCT02432560
Sirolimus	Identification of potential blood and urine markers to evaluate the correct dose of sirolimus	Observational study	Recruiting	NCT03304678
Imatinib mesylate ± co-administration of an mTOR inhibitor	1-month change in serum VEGF-D level	I/II	Recruiting	NCT03131999
Simvastatin co-administered to patients on mTOR inhibitor	Safety over 3 months	I/II (open label)	Recruiting	NCT02061397
Celecoxib (COX-2 inhibitor) as a single agent	Safety and tolerability at 6 months	II (open label)	Recruiting	NCT02484664
Long-term, low dose (1 mg daily) sirolimus	Change in FEV1 at 2 years	III	Recruiting	NCT03150914
Long-term, low dose (1 mg daily) sirolimus	Change in FEV1 at 2 years	III	Recruiting	NCT03150914

COX-2, cyclo-oxygenase-2; FEV1, forced expiratory volume in 1 s; mTOR, mammalian target of rapamycin; VEGF-D, vascular endothelial growth factor-D.

therapy (conditional recommendation based on very low-quality evidence) as treatment for LAM [3▪▪]. Conversely, mTOR inhibitors have shown clinical benefit in patients with LAM; however, the identification of novel targets for alternative therapeutic approaches either in monotherapy or in combination is of outmost importance (Table 2). Statins have been considered for the treatment of LAM owing to their antiproliferative effects and ability to inhibit RhoA GTPase, whose activity is increased in patients carrying *TSC2* mutations [56]. In a retrospective review of patients with LAM treated with simvastatin with sirolimus ($n = 14$), sirolimus alone ($n = 44$), or simvastatin alone ($n = 20$), the combination of sirolimus and simvastatin did not increase the prevalence of drug adverse events or alter the therapeutic effects of sirolimus [57]. A phase 1–2 trial evaluating the safety of simvastatin in LAM patients receiving sirolimus or everolimus is currently ongoing (NCT02061397). Autophagy dysregulation has been implicated in TSC tumorigenesis in case of hyperactive mTOR [58]. Accordingly, inhibition of autophagy by chloroquine or hydroxychloroquine has been proposed as a possible treatment for LAM. A recently concluded phase 1 trial has shown that combination therapy with sirolimus and hydroxychloroquine is effective and well tolerated [46]. Resveratrol, a natural autophagy inducer by direct inhibition of the mTOR-ULK-1 pathway, might represent an additional potential treatment for LAM [59]. LAM lesions are rich in cyclooxygenase-2 (COX-2), while PGE2 are elevated in the serum of LAM patients [60]. A pilot clinical trial of COX-2 inhibitor (celecoxib) in LAM and TSC is currently recruiting participants (NCT02484664). Tyrosine kinases represent another potential therapeutic target. Src kinase inhibitors dasatinib and saracatinib can reduce the migration and invasion of TSC2 (-/-) cells *in vitro* while attenuating their colonization of the lungs in *vivo* [61]. A currently ongoing phase 2 trial will investigate the safety and efficacy of saracatinib in patients with LAM (NCT02737202). KIT tyrosinase kinases may also play a role in the mTOR activation pathways [62]. An ongoing pilot study of imatinib mesylate, a KIT tyrosine kinase inhibitor, will evaluate the changes in VEGF-D serum levels in patients with LAM (NCT03131999). Furthermore, the diffusion of LAM cells to the lung might be prevented by nintedanib, an inhibitor of tumour angiogenesis and lymphangiogenesis by targeting the tyrosine kinase receptors for fibroblast growth factor (FGR), platelet-derived growth factor (PDGF), and vascular endothelial growth factor (VEGF). A pilot study investigating the safety and efficacy of nintedanib in LAM patients is currently ongoing (NCT03062943).

CONCLUSION

Recent years have witnessed major advances in our understanding of the cellular and molecular mechanisms associated with LAM; yet, fundamental questions regarding LAM cell and site of origin remain unanswered. Improved knowledge of disease pathogenesis has helped in developing an effective therapy – sirolimus – which evidence-based guidelines recommend in patients with abnormal/declining lung function; however, sirolimus does not cure LAM and durable benefit requires prolonged treatment. Moreover, safety data on long-term exposure to mTOR inhibitors are limited. Thus, safer and more efficacious treatment strategies are needed. Further development of diagnostic biomarkers will reduce the need for surgical biopsy, while the identification of predictors of disease behaviour and response to treatment will assist physicians with clinical decision-making and facilitate customization of care.

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Conflicts of interest

There are no conflicts of interest.

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