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Optimizing treatments for lymphangioleiomyomatosis

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Abstract

Lymphangioleiomyomatosis (LAM), a multisystem disease predominantly affecting premenopausal women, is associated with cystic lung destruction and lymphatic and kidney tumors. LAM results from the proliferation of a neoplastic cell that has mutations in the tuberous sclerosis complex 1 or 2 genes, leading to activation of a critical regulatory protein, mammalian target of rapamycin. In this report, we discuss the molecular mechanisms regulating LAM cell growth and report the results of therapeutic trials employing new targeted agents. At present, inhibitors of mammalian target of rapamycin such as sirolimus appear to be the most promising therapeutic agents, although drug toxicity and development of resistance are potential problems. As the pathogenesis of LAM is being further recognized, other therapeutic agents such as matrix metalloproteinase inhibitors, statins, interferon, VEGF inhibitors, chloroquine analogs and cyclin-dependent kinase inhibitors, along with sirolimus or a combination of several of these agents, may offer the best hope for effective therapy.

Keywords

chylous effusions; lung function decline; lymphangioleiomyomas; lymphangioleiomyomatosis; sirolimus; tuberous sclerosis complex

Lymphangioleiomyomatosis (LAM) is a multisystem disease, predominantly affecting premenopausal women, that is associated with cystic lung destruction, fluid-filled lymphatic tumors in the axial lymphatics (e.g., lymphangioleiomyomas) and abdominal tumors (e.g., renal angiomyolipomas [AMLs]) [1,2].

LAM frequently presents with dyspnea, pneumothorax, chylothorax, ascites or AML-derived abdominal hemorrhage [1,2]. Imaging studies show numerous thin-walled cysts throughout the lungs, renal AMLs and lymphangioleiomyomas. Pulmonary function tests show reduced expiratory flow rates and/or lung diffusion capacity [1].

The diagnosis of LAM can be confirmed either by a characteristic CT scan and a positive tissue biopsy, a typical CT scan and renal AMLs, chylous effusions, lymphangioleiomyomas or high serum levels of VEGF-D, or a definite diagnosis of tuberous sclerosis complex (TSC) [3].

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LAM results from the proliferation of abnormal smooth muscle-like cells (LAM cells) [2]. Lung lesions comprise clusters of both small spindle-shaped and larger epithelioid LAM cells adjacent to cysts, blood vessels, lymphatics and bronchioles [2]. LAM cells react with antibodies against smooth muscle cell antigens (e.g., smooth muscle α -actin, vimentin and desmin) [2]. The epithelioid cells react with a monoclonal antibody (HMB-45) that recognizes gp100, a premelanosomal protein encoded by the alternatively spliced *Pmel17* gene [2]. The spindle-shaped cells also react with proliferating cell nuclear antigen, which is consistent with these cells being more proliferative [2]. Receptors for estrogen [4,5], progesterone [5], angiotensin II [6], insulin-like growth factors [7], hyaluronic acid (CD44) [8] and chemokines [9] are present in LAM cells.

LAM occurs in approximately a third of women with TSC and sporadically in patients without TSC [1]. TSC is an autosomal-dominant disorder, with variable penetrance, resulting from mutations in the *TSC1* or *TSC2* genes [10] that occur in one in 5800 live births [10]. TSC is associated with hamartoma-like tumors in several organs, cerebral calcifications, seizures, development delay and autism. Sporadic LAM is relatively uncommon; its prevalence has been estimated at 1–2.6/million women [11].

Sporadic LAM is caused by a smooth muscle-like neoplastic cell expressing melanoma proteins in which either the TSC gene 1 (*TSC1*) or 2 (*TSC2*) is mutated [12–15]. Loss of heterozygosity of *TSC2* has been found in LAM lesions from lung and kidney, and LAM cells isolated from lung, blood, chyle and AMLs [12,13,15,16], consistent with Knudson's 'two-hit' hypothesis of tumor development [17]. LAM cells may metastasize. Indeed, identical *TSC2* mutations were found in lung lesions and AMLs from a patient with sporadic LAM, and LAM cells of recipient origin were detected in the donor lung of a transplanted patient [18,19]. LAM cells may be detected in the body fluids (blood, urine, expectorated chyle, pleural or abdominal chylous fluids) of some patients with LAM [15,16]. The source of LAM cells in the lungs has not been determined. Potential sources include AMLs and the lymphatic system [20], but 68% of sporadic patients with LAM do not have evidence of AMLs [21].

Treatment of LAM

Antiestrogen therapy

A role of estrogens in the pathogenesis of LAM is suggested by its female predominance, the frequent occurrence before menopause and reported worsening of lung disease during pregnancy [22], or following the administration of estrogens [23]. Estrogen receptors (ER α) and progesterone receptors (PRs) are expressed by LAM cells [3,4] and AMLs [24].

Estrogen promotes the proliferation of *Tsc*-null rat ELT3 leiomyoma-derived cells *in vitro* and the growth of xenograph subcutaneous tumors *in vivo* [25]. In experimental animals, estradiol was reported to stimulate growth of human AML *TSC2*⁺ cells, which was associated with activation of p42/44 MAPK [26]. Estrogen promotes the survival and pulmonary metastasis of *Tsc* 2^{-/-} ELT3 cells in mice, which was associated with activation of MAPK and inhibited by the MAPK/ERK kinase (MEK) inhibitor CI-1040 [27]. These data suggest that estrogen promotes the survival of circulating TSC2-null cells and their homing to the lung.

ER activation also increased matrix metalloproteinase (MMP)-2 activity of lung-derived cells from an explant from a patient with LAM, promoting cell invasiveness, and doxycycline, an anti-infective with anti-MMP activity, inhibited this effect, suggesting an estrogen-MMP-driven process in lung destruction and LAM cell metastasis [28]. These data

suggest that blockade of the MEK pathway, and inhibition of MMP production and activity, could be new potential approaches to the treatment of LAM.

These data provide a rational for hormonal manipulations in the treatment of LAM. Numerous case reports and occasional uncontrolled studies have documented a beneficial effect of anti-estrogen therapies in LAM. However, no controlled studies have been undertaken to determine their efficacy. In a retrospective study, we found no difference in disease progression between patients treated or not with progesterone [29]. However, there was some evidence for a trend toward decreased rates of functional decline in postmenopausal patients [29], but suppression of ovarian function, by oophorectomy, also did not benefit patients [29]. In one study, treatment with a gonadotrophin-releasing hormone analog failed to prevent decline in lung function in a small group of patients with LAM [30]. However, in a retrospective study, treatment with goserelin was associated with an increase in lung function [31]. Further studies are being considered to determine whether antiestrogen therapy has a role in the treatment of LAM.

A potential new therapeutic approach consists in suppressing estrogen secretion with aromatase inhibitors such as letrozole, especially in postmenopausal women in whom the main source of estrogens are the adrenal glands where aromatase converts androstenedione and testosterone to estrone and estradiol [32]. Letrozole is currently used to treat ER-positive breast cancer. A trial of aromatase inhibition in LAM (TRAIL) is currently underway (Francis X McCormack, P.I., Cincinnati, OH, USA).

mTOR inhibitors

TSC1 and *TSC2* are tumor suppressor genes that encode hamartin and tuberin, respectively [33,34]. Hamartin and tuberin have individual functions and interact to form a cytosolic complex, Hamartin functions in the reorganization of the actin cytoskeleton by interacting with ezrin/radixin/moesin family proteins [35,36]. Tuberin has roles in pathways controlling cell growth and proliferation [36] and is a negative regulator of cell cycle progression. Loss of tuberin shortens the G1 phase of the cell cycle. Tuberin binds p27KIP1, a cyclin-dependent kinase (CDK) inhibitor and by preventing its degradation, leads to inhibition of the cell cycle. In the absence of tuberin, p27 is mislocalized in the cytoplasm, allowing the cell cycle to progress [36].

The TSC1/2 complex acts upstream of the intracellular serine/threonine kinase mTOR and mediates growth factor, energy and stress signals, thereby regulating cell growth and proliferation (Figure 1). There are two different complexes involving mTOR: mTORC1, which also contains raptor (regulatory-associated protein of mTOR), and mTORC2, which contains rictor (rapamycin-insensitive companion of mTOR) [37–39]. TSC2 acts as a GTPase-activating protein for the guanine nucleotide-binding protein Ras homolog enriched in brain (Rheb), promoting the formation of inactive Rheb-GDP from active Rheb-GTP [40–42]. Inhibition of TSC1/2 by growth factor stimulation inhibits GTPase-activating protein activity and allows accumulation of active Rheb-GTP. Rheb-GTP stimulates mTORC1, which phosphorylates substrates such as ribosomal S6 kinase and eukaryotic initiation factor 4E-binding protein, leading to enhanced translation [43].

Both the MAPK and insulin signaling pathways can affect mTORC1 without involving TSC1/2. p90 ribosomal S6 kinase (RSK) can phosphorylate raptor of mTORC1 [44], thus promoting mTORC1 kinase activity directly. Protein kinase B (Akt) phosphorylates proline-rich AKT substrate of 40 kDa (PRAS40), an inhibitor of mTORC1, thereby relieving the inhibition [45]. TSC1/2 positively regulates mTORC2, leading to phosphorylation and activation of Akt [46,47], while it negatively regulates mTORC1. In the presence of growth factors, both the MAPK and insulin signaling pathways can be activated, resulting in

inhibition of TSC1/2 through phosphorylation of TSC2 by RSK, ERK1/2 (extracellular signal-regulated kinase (ERK)-1/2 or Akt [48–52].

Sirolimus is an immunosuppressant that forms a complex with (FK506-binding protein-12 (FKBP-12), which binds and acutely inhibits mTORC1 [53–56]. In one study, sirolimus decreased tumor size in an Eker rat model of TSC with a functionally null germline mutation of *Tsc2*, which spontaneously develops renal cell carcinomas [57]. In a group of patients with TSC or sporadic LAM, who had AMLs, tumor size decreased by half after 1 year of sirolimus therapy, while lung function improved in some patients [58]. Following withdrawal of sirolimus, however, the angioliomas partially regained size. A second report suggested that sirolimus may inhibit the decline in lung function, rather than improve function [59]. Other reports indicate that sirolimus may decrease and resolve chylous effusions in patients with LAM and decrease the size of astrocytomas in TSC patients [60–63].

A multicenter double-blinded sirolimus trial (MILES trial) examined the effect of sirolimus on pulmonary function [64]. Forty-six patients were treated with sirolimus and 43 with placebo for 12 months and followed for an additional year after discontinuation of the study drug or placebo. Study drug dosage was adjusted to maintain a serum level between 5 and 15 ng/ml. Compared with the placebo group, the sirolimus group had improvements from base-line of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV₁), quality of life and functional performance. The absolute difference between mean changes in FEV₁ in both groups was 153 ml, which was highly significant. After discontinuation of sirolimus, lung function decline resumed and paralleled that of the placebo group.

Similar findings were reported in 19 patients treated with sirolimus with either rapidly progressive lung disease or lymphangioleiomyomas and chylous effusions treated with sirolimus [65]. It was found that for approximately 2.5 years prior to therapy, FVC and FEV₁ decreased by 50 ± 30 ml and 100 ± 30 ml per year, respectively. Lung diffusion capacity (DL_{CO}) decreased by 1.1 ± 0.1 ml/mmHg/min per year. Conversely, over 2.6 years of sirolimus therapy, FVC increased by 90 ± 20 ml, FEV₁ increased by 50 ± 20 ml and DL_{CO} increased by 0.2 ± 0.1 ml/mmHg/min. Although pulmonary function responses may have been confounded by concomitant resolution of pleural effusions, in the subgroup of patients without effusions, FEV₁ decreased by 110 ± 10 ml per year before initiation of sirolimus therapy compared with a decrease in only 10 ± 10 ml per year after beginning sirolimus therapy. DL_{CO} decreased by 0.5 ± 0.2 ml/min per mmHg per year before initiation of sirolimus therapy compared with an increase in 0.04 ± 0.1 ml/mmHg/min per year after sirolimus therapy. FVC increased by 40 ± 40 ml per year before initiation of sirolimus therapy compared with an increase in 110 ± 30 ml per year after sirolimus therapy. That is, even in patients without pleural effusions, lung function either stabilized or increased.

During sirolimus therapy, nine patients had complete resolution of their pleural effusions and abdominal lymphangioleiomyomas. These data suggest that sirolimus is highly effective in controlling lymphatic disease in LAM. Interestingly, data from the MILES trial [64] showed that, in contrast to the effect of sirolimus on lung function, serum levels of VEGF-D, a lymphangiogenic growth factor implicated in the pathophysiology of LAM, were reduced even following discontinuation of therapy, suggesting a strong anti-VEGF-D effect.

Thoracic and abdomino-pelvic lymphangioleiomyomas are observed in 16–21% of patients with LAM [2,66]. On CT scans, tumors appear as well-circumscribed masses of variable dimensions, comprising a wall and a central fluid-rich region [66]. These tumor masses are probably caused by the proliferation of LAM cells, leading to compression or obstruction of lymphatic vessels and chylous effusions [67].

Serum VEGF-D, a lymphangiogenic factor, is increased in the serum of patients with LAM compared with normal individuals and appears to be a measure of lymphatic involvement in LAM [68–70]. Hypoxia, which may occur frequently in patients with LAM, is a major stimulus for an increased production of VEGF by cells. Activation of the PI3K/Akt pathway can also increase VEGF secretion independent of hypoxia-related mechanisms [71].

Lymphangioleiomyomas are associated with significant morbidity, pleural effusions that compromise respiratory function, ascites, pain, neuropathy, bladder and rectal compression causing urinary frequency, and tenesmus [67,72–74]. Surgical resection of lymphangioleiomyomas is not recommended in standard care as it may cause lymphatic leakage, chylothorax and ascites. In view of our finding that sirolimus appears to be effective in diminishing the size of lymphangioleiomyomas and chylous effusions [65], we suggest that treatment with sirolimus of patients with chylous effusions and symptomatic lymphangioleiomyomas should be considered before undertaking invasive procedures such as drainage of the fluid or pleurodesis.

Several case reports are consistent with these findings [75,76]. An off-label study undertaken in ten patients with severe LAM treated with sirolimus showed a significant improvement in FVC and FEV₁ after 3 and 6 months of therapy [77].

mTORC1 inhibition also leads to the activation of the Akt and ERK/MAPK signaling pathways [78]. Inhibition of the MAPK pathway along with use of sirolimus has been found to be more efficient at blocking mouse *Tsc2*^{-/-} cell proliferation than either inhibitor alone [79]. Further, inhibition of active Rheb by other means may also be an alternative method of inhibiting LAM cell proliferation or growth. In *Tsc-2* null ELT3 cells, it has been shown that inhibition of Rheb by the Ras inhibitor farnesyl-thiosalicylic acid inhibits proliferation by reducing Rheb and Rheb-GTP, migration and growth of tumors *in vivo* [80].

MMP inhibitors

Since MMPs are present within LAM lesions and may have a role in the pathogenesis of cystic lung destruction in LAM, they could be a potential therapeutic target [81–83]. Doxycycline, an MMP inhibitor that affects growth and migration of neoplastic cells, angiogenesis, lymphangiogenesis and smooth muscle cell growth [84,85], has been shown to inhibit proliferation of ELT3 cells, alter cell morphology and decrease cell adhesion. However, in a study involving AML xenografts, doxycycline did not inhibit MMP gelatinolytic activity or affect subcutaneous tumor size or tumor MMP activity [86]. Although doxycycline did not inhibit proliferation of *Tsc*-null mouse embryonic or human LAM cells, it was shown to reduce the metabolic activity of both cell types [86]. Doxycycline decreased the production of MMP-2 by *Tsc*-null mouse embryonic and human LAM cells, although it had no effect on MMP tissue inhibitors [86]. This effect was not due to a decrease in cell number.

A potential role of doxycycline in the treatment of LAM is suggested in a report of one patient with LAM in whom doxycycline therapy decreased urinary MMP levels and improved lung function [87]. A prospective study undertaken in 34 patients with LAM treated with doxycycline (100 mg/day) for 6 months revealed a decrease in serum and urine levels of MMP-9 and MMP-2 [88]. A potential role of doxycycline, an MMP inhibitor, in the treatment of LAM, is being investigated in the UK in a placebo-controlled trial (Simon Johnson, P.I., Nottingham, UK).

Other potential therapies

Statins

Statins are 3-hydroxy-3-methyl-glutaryl-coenzyme-A reductase inhibitors that may inhibit both sirolimus-sensitive and sirolimus-insensitive mechanisms of tuberlin-null cell growth [89]. In one study, atorvastatin was shown to inhibit the growth of *Tsc2*^{-/-} uterine-derived leiomyoma (ELT-3) and mouse embryonic fibro-blasts cells while impairing Rheb-GTPase activity and function [89]. However, atorvastatin did not decrease tumor size of ethylmtrousou-rea-enhanced renal cystadenoma or spontaneous liver hemangioma in *Tsc2*^{+/-} mice [90]. A synergistic effect of simvastatin and sirolimus was described by Goncharova *et al.* in *Tsc*-null ELT-3 cells and in mice injected with these cells, who develop tumors [91]. Simvastatin inhibited RhoA GTPase activity and proliferation of *Tsc*-null cells by promoting apoptosis [91] and inhibited *Tsc2*-null tumor growth in mice, an effect similar to that of sirolimus. Interestingly, treatment with both drugs prevented recurrence of the tumors even after discontinuation of both drugs and this persistent effect required simvastatin [92]. It appears that simvastatin and sirolimus are synergistic in inhibiting proliferation of *Tsc2*-null cells *in vivo* [92]. Conversely, atorvastatin as a single agent or combined with sirolimus did not increase survival in nude mice bearing *Tsc2*^{-/-} tumors [92]. No correlation between statin use and AML response to sirolimus in patients with TSC or sporadic LAM was observed [58]. Furthermore, in a retrospective study, the rate of decline in lung diffusion for patients on statins was greater than that of their matched controls [93].

Interferon, VEGFs & CDK2 inhibitors

Treatment of *Tsc1*- or *Tsc2*-null cells with IFN- γ was shown to induce apoptosis, and a combination of sirolimus and IFN- γ was reported to be synergistic in causing apoptosis of these cells [94]. IFN- γ expression was absent in AMLs from patients with TSC or LAM, suggesting that the IFN- γ signaling pathway is altered in TSC and LAM [95]. However, the combination of rapamycin and IFN- γ was not found to be more effective than rapamycin alone against TSC-related kidney tumors in *Tsc2*^{+/-} mice [95]. Furthermore, in a subcutaneous *Tsc2*^{-/-} tumor mouse model, sorafenib, a Raf kinase and VEGF pathway inhibitor, and sirolimus together increased survival and decreased tumor volume more effectively than sirolimus alone [92]. IFN- β , which is expressed in LAM tissues and LAM cell cultures, attenuated proliferation of LAM-derived and *Tsc2*-null ELT3 cells, and this effect was potentiated by rapamycin [96].

Although sirolimus inhibits VEGF production, angiogenesis and hypoxia-inducible factor-1, activation of the PI3K/AKT pathway may also increase the secretion of VEGF by both hypoxia-inducible factor-1-dependent and -independent mechanisms [71]. Inhibitors of the PI3K/AKT pathway decrease VEGF secretion. Drugs that block both PI3K and mTOR signaling, shown to inhibit the growth and proliferation of cancer cells [71], could potentially be useful in the treatment of LAM.

Other potential therapeutic options for LAM are CDK2 inhibitors [97–99]. Tuberlin regulates the activity of CDK2, binding to the CDK inhibitor p27, a major regulator of cell cycle progression, preventing its degradation and thereby increasing the amount of p27 bound to CDK2 [98,99]. In tuberlin-negative cells, p27 is degraded as well as delocalized to the cytoplasm [97–99], resulting in lack of inhibition of CDK activities in the cell nucleus by p27. Therefore, inhibitors of CDK2, such as roscovitine, a new potential anticancer agent [99], may have a role in the therapy of LAM.

Finally, mTORC1-independent mechanisms contribute to the survival of tumor cells [100]. Increased AMP-activated protein kinase (AMPK) activity has been reported in TSC tumors and *Tsc*-null cells, and this contributes to the survival of the cells. During sirolimus therapy,

AMPK could contribute to the survival of tumor cells. Rheb can control AMPK activity independently of mTORC1 signaling by reducing p27 levels. Conversely, Rheb depletion upregulates p27 and decreases tumorigenesis [98]. Inhibition of AMPK activity increases p27 levels and correlates with inhibition of CDK2 activity. Targeting Rheb may also be an effective way of reversing TSC and LAM [100].

Inhibitors of autophagy

A major regulator of autophagy is mTORC1 and mTORC2 signaling pathways, and mTOR inhibitors such as sirolimus induce autophagy in tumor cells [101]. Activation of AMPK represses mTOR and may lead to initiation of autophagy. Autophagy is thought to be an important mechanism in tumor suppression, tumor cell survival and cell death [101].

Inhibitors of autophagy may enhance chemotherapy sensitivity of tumors and tumor regression. Chloroquine and its analogs inhibit the growth of cancer cells and induce cell death [102] and may enhance cell killing by chemotherapeutic agents. Several clinical trials have been undertaken in the last few years. Since sirolimus does not inhibit all functions of mTORC1, inhibition of autophagy with chloroquine analogs could potentially result in a decreased LAM cell load in the lungs and improvement in pulmonary function [103].

Immunotherapeutic treatment of LAM

A common feature of LAM cells and melanocytes is that they both express gp100 and melanoma antigens recognized by T cells. Since immunotherapy has been advocated for the treatment of human melanoma, it is reasonable to consider a similar alternative for LAM [104]. Sirolimus, an mTORC1 inhibitor, only affects cell size and proliferation and does not kill LAM cells. As is the case for melanoma cells, tyrosinase proteins (TRP) TRP1 and TRP2 are present in LAM cells. Indeed, cultured LAM cells express gp100, TRP1 and TRP2, and melanosome-like organelles are present in LAM tissue [105]. In addition, immune-cell infiltrates containing CD3, CD4, CD8 and CD11c are also present in LAM tissue. LAM cells also appear to induce T-cell clustering and IFN- γ production by gp100-reactive T cells [105].

Because gp100-positive LAM cells are resistant to anti-proliferative therapies such as sirolimus, these cells could be targeted by immune-mediated mechanisms. Vaccines targeting melanosomal antigens are also a possibility. Conceivably, hyper-proliferative cells in the lungs of patients with LAM may be susceptible to cytotoxic T lymphocyte-mediated immune targeting against melanoma antigens by means of antimelanoma vaccines [105,106]. Consequently, vaccines developed to treat melanoma may also be suitable for the treatment of LAM [106].

Conclusion

In the last 10 years, there has been great progress in understanding the natural history of LAM, its pathogenesis and the biology of the LAM cell. Progress in therapy, however, has been relatively slow. Despite a probable role of estrogens in the pathogenesis of LAM, there is currently no evidence that suppression of estrogen secretion by oophorectomy or GnRH analogs or treatment with progesterone is effective in LAM. However, the rate of decline in lung function appeared to be less in postmenopausal women, suggesting that the severity and rate of progression of disease may be related to endogenous estrogen. At present, inhibitors of regulators of cell proliferation, specifically mTOR inhibitors such as sirolimus or everolimus, appear to be the most promising therapeutic agents, although drug toxicities or development of resistance associated with long-term monotherapy are potential problems.

At present, indications for sirolimus therapy in LAM have yet to be defined. Patients with normal or near-normal lung function should not be treated. Patients with moderate or severe pulmonary disease or patients with large symptomatic chylous effusions and those who show accelerated loss of function should be considered for treatment with sirolimus. In addition, new research suggests that MMP-2 inhibitors, statins, inhibitors of cell cycle progression and VEGF receptors may, in combination with sirolimus, enhance the therapeutic effects of mTORC1 inhibitors.

Expert commentary

In a remarkable example of a direct impact of bench research in the treatment of lung diseases, great progress in the treatment of LAM, a multisystem disease characterized by proliferation of neoplastic smooth muscle-like cells, has recently been reported. The importance of the mTOR signaling pathway in regulating cell growth and proliferation provided an opportunity to test the effect of the mTOR inhibitor sirolimus in LAM. Several studies showed that sirolimus decreases the size of kidney angiomyolipomas, stabilizes and/or improves lung function and dramatically reduces the volume of chylous effusions and lymphangioleiomyomas. Whether resistance to sirolimus will eventually develop is unclear, but it appears that once started, sirolimus therapy cannot be interrupted because of a risk of tumor recurrence or abrupt decline in lung function. Consequently, there is a need for the development of new therapies. Among potential new treatments are MMP inhibitors (doxycycline), statins, anti-VEGF agents, IFN, CDK inhibitors, inhibitors of autophagy (chloroquine) and immunotherapy. We suggest that new trials testing the effectiveness of these therapies are warranted.

Five-year view

New treatments based on inhibition of the MAPK/ERK kinase pathway may be proven to be effective in the future. As a role for lymphangiogenesis and VEGF-D in the pathogenesis of LAM is recognized, it may be possible, as in the case of vascular tumors and malformations, to develop effective antilymphangiogenesis agents in LAM. MMPs inhibitors, statins, IFN, VEGF-D inhibitors and CDK2 inhibitors, along with sirolimus or, as in cancer, a combination of several of these agents, may offer the best hope for effective therapy. More research to discover new treatments is needed.

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- of interest
- of considerable interest

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Key issues

- Lymphangioleiomyomatosis (LAM) is a multisystem disease, predominantly affecting premenopausal women, and is associated with cystic lung destruction and lymphatic and kidney tumors.
- Because of its female predominance and sporadic reports of worsening lung function during pregnancy or administration of estrogens, antiestrogen therapy has been employed to treat LAM but no controlled studies have ever been undertaken.
- Findings that estrogen promotes proliferation of *Tsc*-null cells, growth of xenograph subcutaneous tumors, growth of angiomyolipoma *Tsc2*^{-/-} cells and survival of circulating *TSC2*-null cells suggest an important role of estrogen in the pathogenesis of LAM. Suppression of estrogen secretion with aromatase inhibitors is currently being evaluated.
- The mTOR inhibitor sirolimus decreases the size of kidney angiomyolipomas, improves or stabilizes lung function, and reduces the size of chylous effusions and lymphangioleiomyomas.
- New treatments under consideration are matrix metalloproteinase-2 inhibitors, statins, anti-VEGF agents, IFN- β and - γ , CDK2 inhibitors, inhibitors of autophagy, such as chloroquine, and immunotherapy. None of these treatments have been tested in human subjects so far.
- The low prevalence of LAM makes it unlikely that any of these agents will be tested alone. New trials combining sirolimus with any of these agents are warranted to determine whether the effects are synergistic and lower doses of sirolimus may be employed while enhancing the effects of mTORC1 inhibitors.

Figure 1. Tuberous sclerosis complex integrates multiple signals, such as growth factors, amino acids, AMP and hypoxia, to control cell growth and proliferation

Growth factors stimulate the MAPK and insulin signaling pathways, leading to TSC2 phosphorylation and inactivation. TSC1/2 negatively regulates mTORC1 (containing raptor, DEPTOR, mLST8 and PRAS40) through its actions on Rheb, while it positively regulates mTORC2 (containing rictor, protor [protein observed with rictor-1], mSini, mLST8 and DEPTOR). The insulin signaling pathway can activate mTORC1 without TSC1/2 involvement by Akt-catalyzed phosphorylation of PRAS40, an inhibitor of mTORC1, thereby relieving the inhibition. Similarly, the MAPK signaling pathway can activate mTORC1 without TSC1/2 involvement via RSK-catalyzed phosphorylation of raptor, a component of the mTORC1 complex, leading to mTORC1 activation. Activation of mTORC1 leads to protein translation and to a negative feedback loop on the insulin and MAPK signaling pathways. In the presence of amino acids, the Rag GTPase heterodimers promote the localization of mTORC1 to cellular compartments containing Rheb, thereby facilitating mTORC1 activation. In conditions of low cellular energy or hypoxia, AMPK phosphorylates and activates TSC2, while hypoxia increases the transcription of REDD1, which also activates TSC2, leading to inhibition of translation. Activation of the mTORC1 pathway leads to increases in HIF1 α levels, probably by increasing translation of HIF1 α . HIF1 α activates genes related to hypoxia, such as *VEGF*. TSC2 binds p27KIP1, a cyclin-dependent kinase inhibitor, stabilizing it and resulting in inhibition of cell cycle progression. mTORC2 may have a role in actin dynamics, through activation of PKC α and Rac and Rho GTPases. Sirolimus (rapamycin) inhibits mTORC1, while CI-1040 is a MAPK/ERK inhibitor. Roscovitine is an inhibitor of CDK2, whereas sorafenib is an inhibitor of VEGFR. Simvastatin is an inhibitor of Rho GTPases. IFN- β works with TSC2 to inhibit cell proliferation, probably through cell cycle arrest and apoptosis induction. 4E-BP1: Factor 4E-binding protein 1; Akt: Protein kinase B; AMPK: AMP-activated kinase; CDK2: Cyclin-dependent kinase 2; DEPTOR: Dishevelled, Egl-10 and pleckstrin domain-containing protein 6; ERK: Extracellular signal-regulated kinase; HIF1 α : hypoxia-inducible factor 1, α subunit; MAPK: Mitogen-activated protein kinase; MEK: MAPK/ERK kinase; mTOR: Mammalian target of rapamycin; PI3K: Phosphoinositide-3-kinase; PDK1: Phosphoinositide-dependent protein kinase 1; PKC α : Protein kinase C α ; PRAS40: Proline-

rich AKT substrate of 40 kDa; Rac: Rac GTPase-activating protein; Rag: Ras-related small GTP-binding protein; Raptor: Regulatory-associated protein of mTOR; REDD1: Regulated in the development and DNA damage response 1; Rheb GAP: Ras homolog enriched in brain GTPase-activating protein; Rictor: Rapamycin-insensitive companion of mTOR; RSK: p90 ribosomal S6 kinase; S6K1: S6 kinase 1; TSC: Tuberous sclerosis complex; VEGFR: VEGF receptor.