# Lost in translation: a neglected mTOR target for lymphangioleiomyomatosis

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**Inhibition of rapamycin-insensitive mRNA translation initiation is an underappreciated therapeutic strategy in lymphangioleiomyomatosis.** https://bit.ly/3Oj5Fze

**Cite this article as:** Evans JF, McCormack FX, Sonenberg N, *et al*. Lost in translation: a neglected mTOR target for lymphangioleiomyomatosis. *Eur Respir Rev* 2023; 32: 230100 [DOI: 10.1183/16000617.0100-2023].



Received: 28 May 2023
Accepted: 24 July 2023

## Abstract

Lymphangioleiomyomatosis (LAM) is a cystic lung disease of women resulting from mutations in tuberous sclerosis complex (TSC) genes that suppress the mammalian target of rapamycin complex 1 (mTORC1) pathway. mTORC1 activation enhances a plethora of anabolic cellular functions, mainly *via* the activation of mRNA translation through stimulation of ribosomal protein S6 kinase (S6K1)/ribosomal protein S6 (S6) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)/eukaryotic translation initiation factor 4E (eIF4E). Rapamycin (sirolimus), an allosteric inhibitor of mTORC1, stabilises lung function in many but not all LAM patients and, upon cessation of the drug, disease progression resumes. At clinically tolerable concentrations, rapamycin potently inhibits the ribosomal S6K1/S6 translation ribosome biogenesis and elongation axis, but not the translation 4E-BP1/eIF4E initiation axis. In this mini-review, we propose that inhibition of mTORC1-driven translation initiation is an obvious but underappreciated therapeutic strategy in LAM, TSC and other mTORC1-driven diseases.

## Introduction to lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a pulmonary disease, primarily of women of child-bearing age, but patients may also have multisystemic pathologies including kidney angiomyolipomas, lymphatic involvement and chylous effusions [1]. There is an increasing appreciation of the role of hormonal fluxes in the pathogenesis of LAM, including variations in symptoms during the menstrual cycle and exacerbations associated with pregnancy, exogenous oestrogen use and childbirth [1]. LAM has been reported to reoccur in the donor allograft, indicating the presence of circulating oncogenic LAM stem cells and a metastatic mechanism of the disease [2]. Patients most often present between menarche and menopause with lung collapse or with dyspnoea with exertion. Cystic changes on high-resolution computed tomography and the presence of angiomyolipomas, lymphangioleiomyomas, chylous fluid collections or elevation of the biomarker, vascular endothelial growth factor (VEGF) D, allows for a clinical diagnosis without the need for lung biopsy [1]. LAM occurs both sporadically (SLAM) and in subjects with autosomal-dominant tuberous sclerosis complex (TSC), due to somatic or inherited mutations, respectively, in the TSC genes, TSC1 or TSC2 [1, 3, 4]. TSC-associated LAM is generally discovered at earlier stages than SLAM; most likely due to ascertainment bias introduced through screening of young adult female TSC patients. Next-generation sequencing analyses of laser-captured, micro-dissected LAM lungs revealed that only a fraction of the cells in the LAM lesion harbour TSC mutations and single-cell mRNA sequencing of LAM lungs showed that these small clusters of stem-cell-like and inflammatory TSC mutant cells orchestrate major transcriptional changes in surrounding epithelial, endothelial, fibroblast and immune cells leading to pathological lung parenchymal remodelling [5–7]. A publicly available LAM cell atlas has been constructed using single-cell RNA sequencing LAM lung data from three research groups. This resource allows for convenient exploration of the transcriptome of the LAM lung (https://research.cchmc.org/pbge/lunggens/LCA/LCA.html) [8].

The TSC normally acts as a rheostat, restraining mammalian target of rapamycin complex 1 (mTORC1)-driven cell growth and proliferation when nutrients are scarce, while facilitating these activities in environments abundant with nutrients [9]. As implied by the name, mTORC1 can be allosterically inhibited by the bacterial macrolide rapamycin, which is cytostatic in eukaryotic cells [9]. An outstanding collaboration of scientists, clinicians and LAM patients, culminating in the landmark Multicenter International Lymphangioleiomyomatosis Efficacy and Safety of Sirolimus (MILES) trial [10], showed rapamycin to be safe and to stabilise lung function decline and improve dyspnoea and quality of life in LAM patients [10–12]. The clinical pharmacokinetic and pharmacodynamic profile of rapamycin in MILES subjects (blood levels ∼7 nM) is consistent with the complete inhibition of the mTORC1-driven ribosome biogenesis and mRNA translation elongation axis, but not the mTORC1-driven mRNA translation initiation axis [10, 13]. Rapamycin is hydrophobic and readily partitions into red blood cells and tissues, including a ∼30-fold increase rapamycin levels in the lung over blood [14]. However, even 1000 nM rapamycin does not inhibit the phosphorylation of 4E-BP1$^{Thr37/46}$ and the release of rate-limiting eukaryotic translation initiation factor (eIF4E) that drives translation of mRNAs coding for proliferative, anti-apoptopic and pro-angiogenic proteins [15, 16].

## Rapamycin, mTOR and TSC

Pharmaceutical scientists isolated rapamycin from *Streptomyces hygroscopicus* bacteria sourced from Rapa Nui (Easter Island). Initially recognised for its potential as an antifungal agent, it was subsequently identified as an immunosuppressant and, later, as an inhibitor of cell growth [9]. The protein target of rapamycin was first identified in yeast as a phosphatidylinositol-3-kinase (PI3K) family serine-threonine kinase that bound to rapamycin through an intermediate protein, the immunophilin FK506-binding protein 12 (FKBP12). Based on these discoveries in yeast, two mammalian complexes containing the mammalian equivalent protein termed mTOR were discovered, namely mTORC1 and mTORC2. FKBP12 binds to rapamycin and then docks onto the mTOR kinase site in mTORC1 but not mTORC2 [9]. The mTORC1 complex consists of several proteins including mTOR kinase and the adaptor protein named regulatory-associated protein of mTOR (Raptor) that recruits the substrates for phosphorylation by mTOR. mTORC1 activation results in phosphorylation of proteins that enhance anabolic metabolism and reduce catabolic metabolism in cells [9, 17]. The TSC1/TSC2 complex constitutes a major integration node for the mTORC1 growth pathway by acting as a GTPase for Ras homologue enriched in brain (Rheb), another GTPase that regulates mTORC1 [17]. The TSC complex integrates multiple inputs from many kinase pathways within the cell, particularly through adenosine monophosphate-activated protein kinase, which senses the ratio of ATP to AMP. The TSC complex resides mainly on the lysosome surface and is regulated by the availability of amino acids. The loss of TSC function eliminates the tonic repression of Rheb, resulting in constitutive mTORC1 activation, and disrupts the elegant orchestration of cellular metabolic pathways by the pathway. This ability of the cell to sense environmental conditions is largely absent in LAM TSC-deficient cells [1].

mTORC1 controls protein biosynthesis by regulating mRNA translation through two axes, namely ribosomal protein S6 kinase beta-1 (S6K1)/ribosomal protein S6 (S6) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1)/eukaryotic translation initiation factor 4E (eIF4E) [17, 18]. Phosphorylation of S6K1 by mTORC1 mainly controls ribosomal biosynthesis and cell size, whereas mTORC1 phosphorylation of 4E-BP1 primarily regulates proliferation, angiogenesis and stem cell survival [9, 18]. mTORC2 also contains several proteins in addition to the mTOR kinase, including the mTORC2-specific protein named rapamycin-insensitive companion of mammalian target of rapamycin (Rictor), which sterically prevents the binding of FKBP12 and rapamycin to this complex [19]. Activation of mTORC2 modulates important aspects of insulin/PI3K signalling, cytoskeletal remodelling and cell survival, as has been recently reviewed [20]. Although the TSC complex does not directly regulate mTORC2, there are feedback mechanisms between mTORC1 and mTORC2 [20]. In fact, in LAM TSC2-null cells, silencing of RNA to Raptor or Rictor has been shown to inhibit DNA synthesis [21]. Expression of genes in the mTOR pathways are commonly increased in many cancers and orchestrate metabolic reprogramming to meet the anabolic demands of rapidly proliferating cancer cells.

Since the initial discovery of mTOR, much has been learned about the regulation of the mTORC1 major cellular growth pathway and its role in protein synthesis. However, published illustrations of the mTORC1 pathway often still incorrectly depict equal inhibition of both axes of mRNA translation by rapamycin, despite its limited capacity for 4E-BP1/EIF4E inhibition [18]. Although rapamycin inhibits phosphorylation of several sites on 4E-BP1, it does not inhibit the phosphorylation of 4E-BP1$^{Thr37/46}$, which is required for release of the rate-limiting translation initiation factor EIF4E. The key phosphorylated protein residues in the mTOR pathways that are sensitive or insensitive to rapamycin inhibition are shown in figure 1.

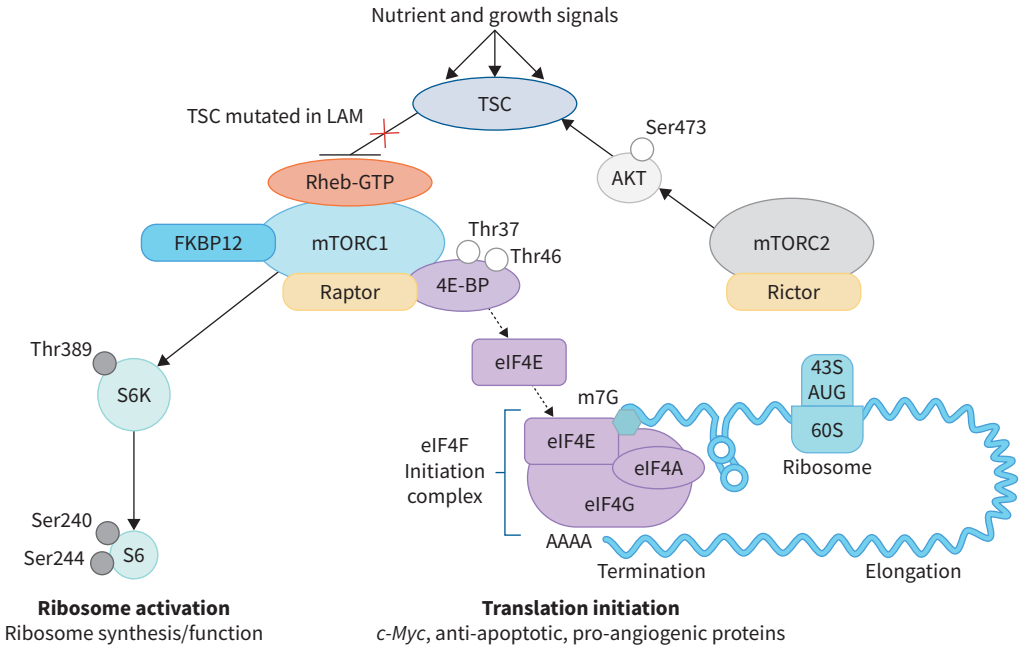


**FIGURE 1** Simplified mammalian target of rapamycin (mTOR) pathways highlighting protein synthesis initiation. The TSC responds to external growth signals to regulate the major mTORC1 growth pathway. Rapamycin binds to the immunophilin FKBP12, which then binds to mTORC1 to inhibit S6K/S6 ribosome synthesis and mRNA elongation but not 4E-BP/eIF4E mRNA translation initiation. In LAM cells, mutations in TSC genes result in de-repression of the GTPase Rheb and hyperactivation of mTOR in mTORC1. Ribosome biogenesis and function are regulated by the kinase S6K1 and its substrate S6, which are activated by the Raptor-guided, rapamycin-sensitive phosphorylation at $S6K1^{Thr389}$, which in turn phosphorylates the ribosome protein $S6^{240/244}$. Nonphosphorylated 4E-BP binds the oncogenic initiation factor eIF4E. Rapamycin-insensitive phosphorylation of 4E-BP1 by mTORC1 on $Thr^{37/46}$ results in the release of eIF4E from 4E-BP1. eIF4E binds to the scaffold protein eIF4G and, along with eIF4A, forms the eIF4F translation initiation complex. eIF4E binds to 5′ methyl-7-G-capped (m7G) mRNAs and the helicase eIF4A unwinds the secondary mRNA structure. Highly eIF4E-dependent translation includes mRNAs for Myc, proliferative, anti-apoptotic and pro-angiogenic proteins. Rictor in mTORC2 prevents rapamycin inhibition of mTOR in this complex and mTOR in this complex phosphorylates many proteins in a rapamycin-insensitive fashion, including AKT $Ser^{473}$, which is a biomarker of mTORC2 activity. White balls indicate rapamycin-insensitive phosphorylation sites; black balls represent rapamycin-sensitive phosphorylation sites. The blue helical and wavy line represents eIF4E-dependent mRNAs. 4E-BP: eukaryotic initiation factor 4E-binding protein; eIF4E: eukaryotic translation initiation factor 4E; FKBP: FK506-binding protein; GTP: guanosine diphosphate; LAM: lymphangioleiomyomatosis; mTORC: mammalian target of rapamycin complex; Raptor: regulatory-associated protein of mTOR; Rheb: Ras homologue enriched in brain; Rictor: rapamycin-insensitive companion of mammalian target of rapamycin; S6: ribosomal protein S6; S6K: ribosomal protein S6 kinase; TSC: tuberous sclerosis complex.

## Control of mRNA translation initiation by mTORC1

The primary control of mRNA translation initiation is through mTORC1-mediated phosphorylation of 4E-BPs, which releases the initiation factor eIF4E, the rate-limiting initiation protein that binds to the m7G untranslated (5′UTR) cap structure of mRNAs [22]. All eukaryotic cellular mRNAs translate in an m7G-cap-dependent manner. Selective mRNAs with extensive 5′ secondary structures, terminal oligopyrimidine sequences or long 5′UTRs, such as those coding for c-Myc, B-cell lymphoma 2, B-cell lymphoma-extra large, VEGF-C, myeloid cell leukaemia 1, matrix metallopeptidase 9, oestrogen receptor α (ER-α) and transcription factor EB, are particularly dependent on increased eIF4E. Global protein expression is less sensitive to eIF4E expression and the eIF4E heterozygous knockout mouse does not have a pathological phenotype [23]. eIF4E, the least abundant of the initiation factors, complexes with the scaffold protein eIF4G and the helicase eIF4A to form the eIF4F initiation complex (figure 1). 4E-BPs compete with the eIF4F complex scaffold protein eIF4G for binding to eIF4E. After eIF4F is assembled on the m7G-cap, the 43S preinitiation ribosome complex binds to the mRNA, followed by scanning of the 5′ UTR until the AUG initiation codon is encountered and protein elongation begins.

There are three mammalian 4E-BP proteins with 4E-BP1 being the most abundant in many cell types. Stepwise phosphorylation of 4E-BPs by mTORC1 occurs with the $Thr^{37}$ and $Thr^{46}$ residues priming the subsequent phosphorylations [13, 22]. Elegant solution nuclear magnetic resonance spectroscopy studies of 4E-BP1/eIF4E in complex with mTORC1 have demonstrated that, in the presence of Raptor, rapamycin recruits FKBP12 to the binding domain of mTOR to sterically occlude most of the kinase substrate sites [24]. However, the early 4E-BP1 $Thr^{37/46}$ sites are still accessible and can be phosphorylated while attached to mTORC1. Cell type-dependent sensitivities to rapamycin are determined in part by the ratio of different 4E-BPs. For instance, 4E-BP1 is the most abundant 4E-BP in fibroblasts and rapamycin does not inhibit the 4E-BP1/eIF4E axis. However, in brain cells and some lymphocytes, 4E-BP2 is the major 4E-BP and those cell types appear to be more susceptible to allosteric mTORC1 inhibitors.

In many cancer cells, increased eIF4E expression has been associated with tumour growth, while housekeeping genes are less dependent on eIF4E [22]. However, it has been suggested that the critical variable to control of initiation of translation may be the ratio of 4E-BP/eIF4E. The anti-proliferative consequences of inhibiting the 4E-BP1/eIF4E axis in cancers has been suggested to be mainly through a c-Myc-eIF4E feedforward loop [22]. eIF4E is phosphorylated by MAP kinase-interacting serine/threonine protein kinases 1/2 (MNK1/2) and, in some tumour cells, inhibition of MNK1/2 inhibits growth [25]. Overexpression of eIF4E itself through the eIF4F complex can be oncogenic [22]. eIF4E, eIF4G and eIF4A, the three components of the IF4F translation initiation complex, are all highly expressed in LAM lung and angiomyolipoma stem-like cells (Y. Tang, Brigham and Women's Hospital, Harvard, Boston, USA; personal communication).

## mTORC1 translation pathways in LAM

The initial characterisation of the pathway downstream of the TSC mutation in LAM revealed pS6K activation [26]. In this landmark paper, it was clearly shown that TSC2 transfected into LAM smooth muscle cells partially inhibited phosphorylation of S6K1 and that rapamycin inhibited DNA synthesis *in vitro* but was cytostatic rather than cytotoxic. Of particular interest regarding LAM, other studies have shown that the oestrogen receptor is phosphorylated in a rapamycin-sensitive manner by S6K1 [27] and that oestrogen activates LAM cell growth *in vitro* [28]. Translation of ER-α is also highly dependent on eIF4E translation initiation [22].

There are no published studies in LAM or LAM-associated cells on the distribution of different phosphorylated isoforms of pS6Ks or 4E-BPs. However, in a study of 30 LAM lung biopsies, the distribution of p4E-BP1 *versus* pS6K1 and pS6 demonstrated that there was high expression of all three phosphorylated proteins in LAM bronchi and lung parenchyma [29]. In four LAM lung fibroblast lines grown *in vitro*, we showed that pS6, but not p4E-$BP1^{Thr37/46}$, was potently inhibited by 10 nM rapamycin [30]. In contrast, the dual PI3K/mTOR kinase inhibitor omipalisib inhibited both axes, albeit much more potently (~100-fold) for pS6 than p4E-BP1 [30].

It is of interest that, in fibroblasts from normal human lungs and from patients with idiopathic pulmonary fibrosis, transforming growth factor-β1 induction of collagen, type I, alpha 1 and actin alpha 2 (protein product α-smooth muscle actin) mRNA was shown to occur *via* the 4E-BP axis [31, 32]. An increase in fibrotic genes has been demonstrated in LAM unique cells and in a subpopulation of fibroblasts in the LAM lung [5–7]. If this increase in fibrotic proteins has pathological consequences in LAM, this may represent another beneficial outcome of inhibiting the 4E-BP1/eIF4E axis in the disease.

## Translation initiation as a therapeutic target in LAM

Rapamycin (sirolimus) and its close analogues (rapalogs), everolimus and temsirolimus, are United States Food and Drug Administration approved first-generation mTORC1 inhibitors (table 1). These drugs potently block the S6K1/S6 translation axis but not the 4E-BP1/eIF4E axis. In addition, they do not inhibit mTORC2. In aggressive cancers, monotherapy with rapalogs has been disappointingly ineffective [33]. In some specific cancers, including triple-negative breast cancers where the Rictor gene is amplified, the inhibition of mTORC2 activity *via* antisense Rictor in combination with lapatibinib has been shown to effectively inhibit tumour growth in preclinical studies [34].

As the importance of inhibiting the 4E-BP1/eIF4E axis in hyperactive mTORC1-driven cancers became better appreciated, second-generation catalytic site PI3K/mTOR kinase and dual mTORC1/mTORC2 mTOR inhibitors were developed [16, 35]. At clinically tolerable concentrations, despite potently inhibiting the S6K1/S6 translation node, catalytic mTOR kinase inhibitors do not inhibit phosphorylation of the p4E-BP $Thr^{37/46}$ residues, so they do not inhibit the release of eIF4E or translation initiation of the highly eIF4E-dependent proliferative, anti-apoptotic and pro-angiogenic mRNAs [20, 36].

**TABLE 1** Nonexhaustive list of mammalian target of rapamycin (mTOR) inhibitors and selective translation initiation inhibitors

| mTORC1 allosteric | PI3K/mTOR catalytic | Combination allosteric/catalytic | Translation initiation |
|---|---|---|---|
| Sirolimus[#] | Alpelisib[#] | RapaLink-1[+] | Zotatifin[¶] |
| Everolimus[#] | Omipalisib[¶] | RMC-5552[¶] | Tomivosertib[¶] |
| Temsirolimus[#] | Vistusertib[¶] | | 4EGI-1, EGPI-1[+] |

mTORC1: mammalian target of rapamycin complex 1; PI3K: phosphatidylinositol-3-kinase. [#]: Marketed drug; [¶]: clinical trials; [+]: preclinical development.

Third-generation mTOR inhibitors termed RapaLinks were developed in which rapamycin is covalently linked to catalytic mTOR kinase inhibitors [37, 38]. Additional modification of the rapamycin macrolide and changes in the linker distance between the mTOR kinase catalytic inhibitor resulted in bi-steric mTOR inhibitors, such as RMC-5552 [39, 40], that inhibit both the S6K1/S6 and 4E-BP1/eIF4E axes but not mTORC2. Some of these third-generation mTOR inhibitors are in early phase 1/2 clinical trials in advanced cancers and the bi-steric inhibitor RMC-5552 reaches blood concentrations which should inhibit the mTORC1-driven 4E-BP1/eIF4E translation initiation axis [41, 42]. These compounds may be

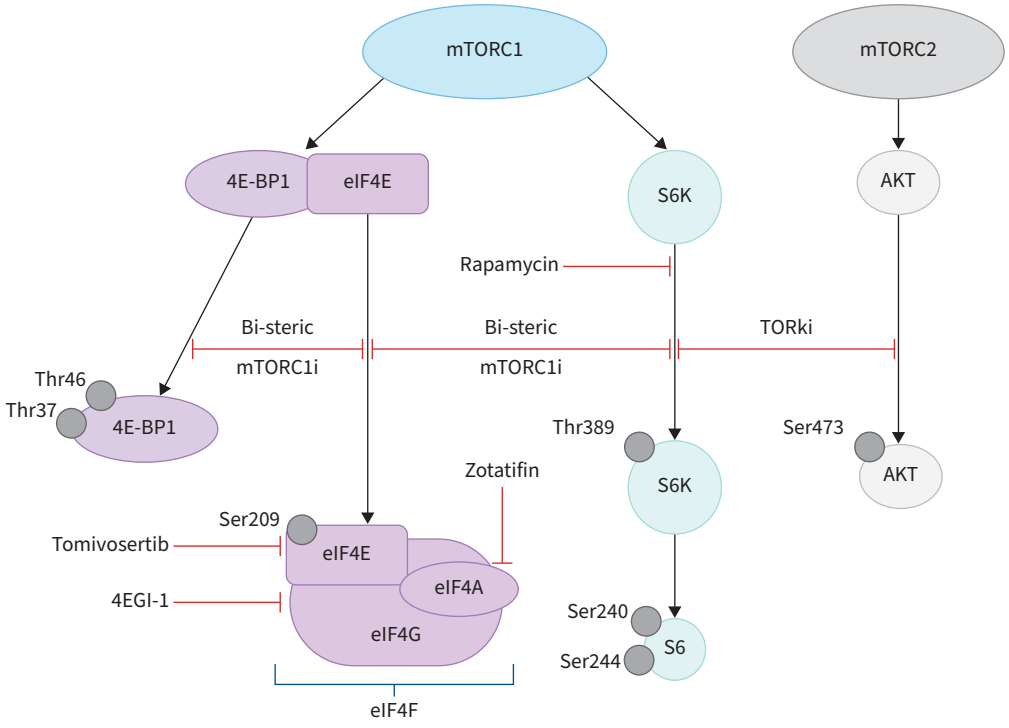


**FIGURE 2** Simplified inhibition profile of mammalian target of rapamycin (mTOR) and translation initiation inhibitors at clinically tolerable concentrations. Activation of mammalian target of rapamycin complex (mTORC) 1 results in mTOR kinase phosphorylation of many substrates including ribosomal protein S6 kinase (S6K) and eukaryotic initiation factor 4E-binding protein 1 (4E-BP1). mTORC2 substrates are more limited but include AKT. Rapamycin and rapalogs inhibit phosphorylation of S6K and its many substrates including the ribosomal protein S6. At clinically tolerated concentrations mTOR kinase inhibitors (TORki) inhibit S6K/S6 and AKT. Bi-steric mTORC1 inhibitors (mTORC1i) inhibit S6K and 4E-BP1 phosphorylation but some, but not all, do not inhibit phosphorylation of AKT. Translation initiation inhibitors modify the efficacy of the three components of the eukaryotic translation initiation factor (eIF) 4F translation initiation complex, namely eIF4E, eIF4G and eIF4A. 4EGI-1 blocks eIF4G binding eIF4E, the mRNA cap binding protein, thus preventing eIF4E-dependent mRNA translation. The MNK kinase-interacting serine/threonine protein kinase inhibitor tomivosertib inhibits phosphorylation of eIF4E decreasing its binding to some mRNAs. The eIF4A helicase inhibitor zotatifin prevents unwinding of the secondary structure of eIF4E-dependent mRNAs thus preventing their translation. Grey circles indicate key phosphorylation sites.

particularly beneficial in cancers with loss of TSC2, such as in peripheral epithelioid cell tumours [43]. It should be noted that the risk/benefit considerations are different for LAM than for more aggressive neoplasms, since through clinical trials and excellent clinical care, LAM has become a chronic disease with an effective therapy and a median survival that is measured in decades [1]. For use in most LAM patients, the safety profile of these inhibitors will need to be equal to or better than that of rapamycin itself. An important ongoing clinical trial, the Multicenter Interventional Lymphangioleiomyomatosis (LAM) Early Disease Trial (MILED), is assessing the efficacy of early, low-dose (1 mg daily) rapamycin treatment in LAM patients with normal lung function (NCT03150914). From a pharmacodynamic perspective, the standard 2 $mg \cdot day^{-1}$ and 1 $mg \cdot day^{-1}$ rapamycin dosing protocols would be predicted to fully inhibit the S6K1/S6 translation axis in LAM cells, but to have no effect on the 4E-BP1/eIF4E translation initiation axis.

Compounds that specifically directly target mRNA translation initiation and proteins in the eIF4F translation initiation complex are also in preclinical and clinical development [22]. Cap-competitive inhibitors of eIF4E based on a nucleotide scaffold have been shown to inhibit cell tumour growth *in vitro* [44]. Another class of translation initiation inhibitors blocks the binding of eIF4G to eIF4E including the compounds 4EGI-1, EGPI-1 and SBI-756, which inhibited tumour growth in preclinical studies [22, 45]. MNK inhibitors that suppress phosphorylation of the oncogene eIF4E have also been developed, including tomivosertib, which is in phase I clinical oncology trials [25]. The eIF4A helicase translation initiation inhibitor zotatifin has shown efficacy *in vivo* in receptor kinase-driven tumours and benefit in phase 1/2 oestrogen receptor positive metastatic breast cancer in combination with fulvestrant and abemaciclib [46, 47]. Figure 2 illustrates the main sites of action of mTOR pathway inhibitors at clinically tolerable concentrations.

## Conclusions

In this mini-review we propose that inhibition of mTORC1-driven mRNA translation inhibition is an obvious but heretofore untapped therapeutic target in LAM. Selective translation initiation inhibitors and/or bi-steric mTORC1 inhibitors that inhibit both the ribosomal and the initiation translation axes hold considerable promise as anti-proliferative strategies in LAM, TSC and other mTORC1-driven diseases.

Provenance: submitted article, peer reviewed.

Acknowledgements: The authors thank LAM patients for the donation of LAM tissues, Kevin Clark for artistic help with figures 1 and 2, and Akansha Goel for careful review of the manuscript.

Author contributions: All authors contributed to the writing of this mini-review, and approved the final version.

Conflict of interest: No author has a conflict of interest.

Support statement: NIH/NHLBI RO1 HL141462, RO1 HL158737, RO1151647, and U01HL131022 to V.P. Krymskaya; UO1HL131755 and UO1HL13102 to F.X. McCormack; Canadian Institutes of Health Research Foundation grant to N. Sonenberg. Funding information for this article has been deposited with the Crossref Funder Registry.

## References

1 McCarthy C, Gupta N, Johnson SR, *et al.* Lymphangioleiomyomatosis: pathogenesis, clinical features, diagnosis, and management. *Lancet Respir Med* 2021; 9: 1313–1327.
2 McCormack FX, Travis WD, Colby TV, *et al.* Lymphangioleiomyomatosis: calling it what it is: a low-grade, destructive, metastasizing neoplasm. *Am J Respir Crit Care Med* 2012; 186: 1210–1212.
3 Carsillo T, Astrinidis A, Henske EP. Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. *Proc Natl Acad Sci USA* 2000; 97: 6085–6090.
4 Rebaine Y, Nassar M, Girard B, *et al.* Tuberous sclerosis complex for the pulmonologist. *Eur Respir Rev* 2021; 30: 200348.
5 Guo M, Yu JJ, Perl AK, *et al.* Single cell transcriptomic analysis identifies a unique pulmonary lymphangioleiomyomatosis cell. *Am J Respir Crit Care Med* 2020; 202: 1373–1387.
6 Obraztsova K, Basil M, Rue R, *et al.* mTORC1 activation in lung mesenchyme drives sex- and age-dependent pulmonary structure and function decline. *Nat Commun* 2020; 11: 5640.
7 Tang Y, Kwiatkowski DJ, Henske EP. Midkine expression by stem-like tumor cells drives resistance to mTOR inhibition and an immune-suppressive microenvironment. *Nat Commun* 2022; 13: 5018.
8 Du Y, Guo M, Wu Y, *et al.* LAM cell atlas. *Thorax* 2023; 78: 85–87.
9 Wullschlerger S, Loewith R, Hall MN. TOR signaling in growth and metabolism. *Cell* 2006; 124: 471–484.

10 McCormack FX, Inoue Y, Moss J, *et al.* Efficacy and safety of sirolimus in lymphangioleiomyomatosis. *N Engl J Med* 2011; 364: 1595–1606.
11 Bee J, Fuller S, Miller S, *et al.* Lung function response and side effects to rapamycin for lymphangioleiomyomatosis: a prospective national cohort study. *Thorax* 2018; 73: 369–375.
12 Gupta N, Lee HS, Young LR, *et al.* Analysis of the MILES cohort reveals determinants of disease progression and treatment response in lymphangioleiomyomatosis. *Eur Respir J* 2021; 53: 1802066.
13 Mahalati K, Kahan BD. Clinical pharmacokinetics of sirolimus. *Clin Pharm* 2001; 40: 573–585.
14 Napoli KL, Wang M-E, Stepkowski SM, *et al.* Distribution of sirolimus in rat tissue. *Clin Biochem* 1997; 30: 135–142.
15 Gingras AC, Gygi SP, Raught B, *et al.* Regulation of 4E-BP1 phosphorylation: a novel two-step mechanism. *Genes Dev* 1999; 13: 1422–1437.
16 Mortensen DS, Fultz KE, Xu S, *et al.* CC-223, a potent and selective inhibitor of mTOR kinase: *in vitro* and *in vivo* characterization. *Mol Cancer Res* 2015; 14: 1295–1305.
17 Tee AR, Manning BD, Roux PP, *et al.* Tuberous sclerosis complex gene products, tuberin and hamartin, control mTOR signaling by acting as a GTPase-activating protein complex toward Rheb. *Curr Biol* 2003; 13: 1259–1268.
18 Choo AY, Yoon SO, Kim SG, *et al.* Rapamycin differentially inhibits S6 K and 4E-BP1 to mediate cell type specific repression of mRNA. *Proc Natl Acad Sci USA* 2008; 105: 17414–17419.
19 Yu Z, Chen J, Takagi E, *et al.* Interactions between mTORC2 core subunits Rictor and mSin1 dictate selective and context-dependent phosphorylation of substrate kinase SGK1 and AKT. *J Biol Chem* 2022; 2: 102288.
20 Fu W, Hall MN. Regulation of mTORC2 signaling. *Genes* 2020; 11: 1045.
21 Goncharova EA, Goncharova DA, Li H, *et al.* mTORC2 is required for proliferation and survival of Tsc2-null cells. *Mol Cell Biol* 2011; 31: 2484–2498.
22 Pelletier J, Sonenberg N. Therapeutic targeting of eukaryotic initiation factor eIF4E. *Biochem Soc Trans* 2023; 51: 113–124.
23 Truitt ML, Conn CS, Shi Z, *et al.* Differential requirements for eIF4E dose in normal development and cancer. *Cell* 2015; 162: 59–71.
24 Bohm R, Imseng S, Jakob RP, *et al.* The dynamic mechanism of 4E-BP1 recognition and phosphorylation by mTORC1. *Mol Cell* 2021; 81: 2403–2416.
25 Jin X, Yu R, Wang X, *et al.* Progress in developing MNK inhibitors. *Eur J Med Chem* 2021; 219: 113420.
26 Goncharova EA, Goncharova D, Eszterhas A, *et al.* Tuberin regulates p70 S6 kinase activation and ribosomal protein S6 phosphorylation: a role for the TSC2 tumor suppressor gene in pulmonary lymphangioleiomyomatosis (LAM). *J Biol Chem* 2002; 277: 30958–30967.
27 Yamnik RL, Digilova A, Davis DC, *et al.* S6 kinase 1 regulates estrogen receptor $\alpha$ in control of breast cancer cell proliferation. *J Biol Chem* 2009; 284: 6361–6369.
28 Yu J, Astrinidis A, Howard S, *et al.* Estradiol and tamoxifen stimulate LAM-associated angiomyolipoma cell growth and activate both genomic and non-genomic signaling pathways. *Am J Physiol Lung Cell Mol Physiol* 2004; 286: 694–700.
29 Hayashi T, Kumasaka T, Mitani, K, *et al.* Bronchial involvement in advanced stage lymphangioleiomyomatosis: histopathologic and molecular analyses. *Hum Pathol* 2016; 50: 34–42.
30 Evans JF, Rue RW, Mukhitov AR, *et al.* Inhibition of growth of TSC2-null cells by a PI3K/mTOR inhibitor but not by a selective MNK1/2 inhibitor. *Biomolecules* 2020; 10: 28.
31 Woodcock HV, Ely JD, Guillotin D, *et al.* The mTORC1/4E-BP1 axis represents a critical signaling node during fibrogenesis. *Nat Commun* 2019; 10: 6.
32 Plate M, Guillotin D, Chambers RC. The promise of mTOR as a therapeutic target pathway in idiopathic pulmonary fibrosis. *Eur Respir Rev* 2020; 29: 200269.
33 Li J, Kim SG, Blenis J. Rapamycin, one drug, many effects. *Cell Metab* 2014; 19: 373–379.
34 Werfel TA, Wang S, Jackson MA, *et al.* Selective mTORC2 inhibitor therapeutically blocks breast cancer cell growth and survival. *Cancer Res* 2018; 78: 1845–1858.
35 Morscher RJ, Brard C, Berlanger P, *et al.* First-in-child phase I/II study of the dual mTORC1/2 inhibitor vistusertib (AZD2014) as monotherapy and in combination with topotecan-temozolomide in children with advanced malignancies: arms D and F of the AcSe-ESMART trial. *Eur J Cancer* 2021; 157: 2268–2277.
36 Ducker GS, Atreya CE, Simko JP, *et al.* Incomplete inhibition of phosphor 4E-BP primary resistance to ATP-competitive mTOR inhibitors. *Oncogene* 2014; 33: 1590–1600.
37 Rodrik-Outmezguine VS, Okaniwa M, Yao Z, *et al.* Overcoming mTOR resistance mutations with a new generation of mTOR inhibitor. *Nature* 2016; 534: 272–276.
38 Lee BJ, Boyer JA, Burnett GL, *et al.* Selective inhibitors of mTORC1 activate 4E-BP1 and suppress tumor growth. *Nat Chem Biol* 2021; 17: 1065–1074.
39 Burnett GL, Yang YC, Aggen JB, *et al.* Discovery of RMC-5552, a selective bi-steric inhibitor of mTORC1 for the treatment of mTORC1-activated tumors. *J Med Chem* 2023; 66: 149–169.

40 Mahauad-Fernandez WD, Yang YC, Lai I, *et al.* Bi-steric mTORC1-selective inhibitors activate 4E-BP1, suppress MYC. Restore anti-tumor immunity, and co-operate with immune checkpoint inhibition to elicit tumor regression. *Cancer Res* 2023; 83: LB015.

41 Burris H, Ulahannan SV, Haura E, *et al.* The bi-steric mTORC1-selective inhibitor RMC-552 in tumors with activation of mTOR signaling. Preclinical activity in combination with RAS (ON) inhibitors in RAS-addicted tumors and initial clinical findings from a single agent phase 1/1b study (NCT04774952). *ASCO* 2022; 2022: 3098. https://s3.us-west-2.amazonaws.com/rvmdpubs.revmed.com/2022/ASCO_2022_RMC5552-001.pdf

42 Wagner AJ, Ravi V, Reidel RF, *et al.* Sirolimus for patients with malignant perivascular epithelioid cell tumors. *J Clin Oncol* 2021; 39: 3660–3670.

43 Sekiyama N, Arthanari H, Papadopoulos E, *et al.* Molecular mechanism of the dual activity of 4EGI-1: dissociating eIF4G from eIF4E but stabilizing the binding of unphosphorylated 4E-BP1. *Proc Natl Acad Sci USA* 2015; 112: E4036–E4045.

44 Cárdenas EL, O'Rourke RL, Menon A, *et al.* Design of cell permeable inhibitors of eukaryotic translation initiation factor (eIF4E) for inhibiting cap-dependent aberrant cap-dependent translation in cancer. *bioRxiv* 2023; preprint [https://doi.org/10.1101/2023.05.23.541912].

45 Herzog LO, Walters B, Buono R, *et al.* Targeting eIF4F translation initiation complex with SBI-756 sensitizes B lymphoma cells to venetoclax. *Br J Cancer* 2021; 124: 1098–1102.

46 Gerson-Gurwitz A, Young NP, Goel VK, *et al.* Zotatifin, an eIF4A-selective inhibitor blocks tumor growth in receptor kinase-driven tumors. *Front Oncol* 2021; 11: 766298.

47 Rosen E. Phase 1/2 dose expansion study evaluating first-in-class eIF4A inhibitor zotatifin in patients with ER+ metastatic breast cancer. *J Clin Oncol* 2023; 41: Suppl., 1080.