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New Therapies for LAM

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Conflict of interest disclosures

Boehringer Ingelheim, Roche, AstraZeneca

LAM treatment: where we are now?

Use of sirolimus for treatment of LAM is recommended in the presence of at least one of this criteria:

- ✓ FEV1 ≤ 70% at baseline
- ✓ Chylous
- ✓ Rapid decline in lung function (change in FEV1 >90 mL/year)
- ✓ Need for supplemental oxygen

Sirolimus has been approved for LAM patients by the US Food and Drug Administration in 2015 and European Medicines Agency in 2016

McCormack FX et al N Engl J Med 2011
McCormack FX et al, ATS/JRS guide lines AJRCCM 2016

LAM treatment: where we are now?

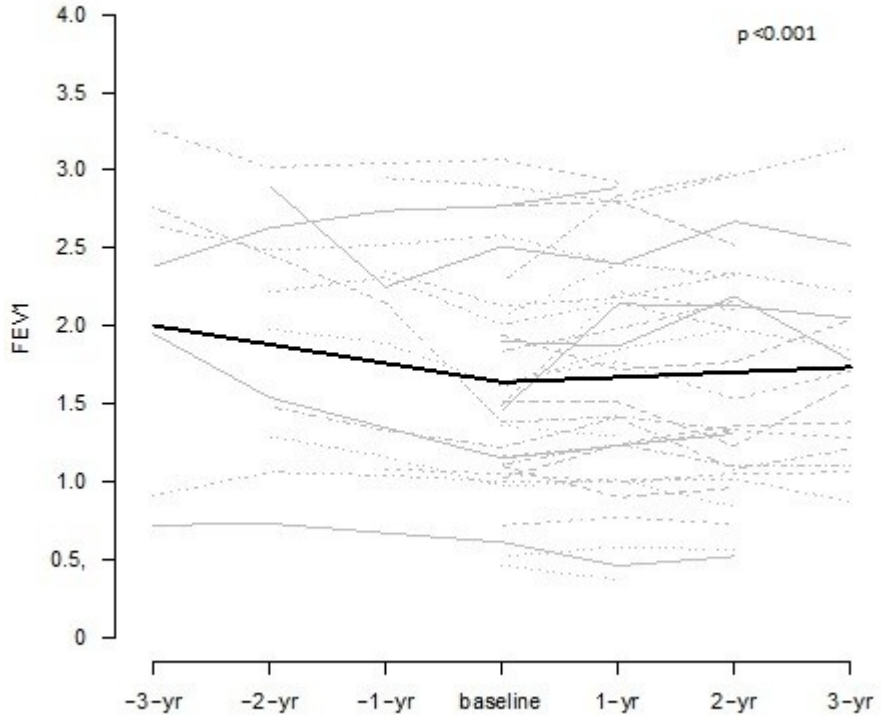
Use of everolimus for treatment of TSC-LAM is recommended in the presence of at least one of this criteria:

- ✓sub-ep … ntervention, but are …
- ✓Renal angiomyolipomas at risk of complications (based on factors such as tumour size or presence of aneurysm, or presence of multiple or bilateral tumours), but that do not require immediate surgery

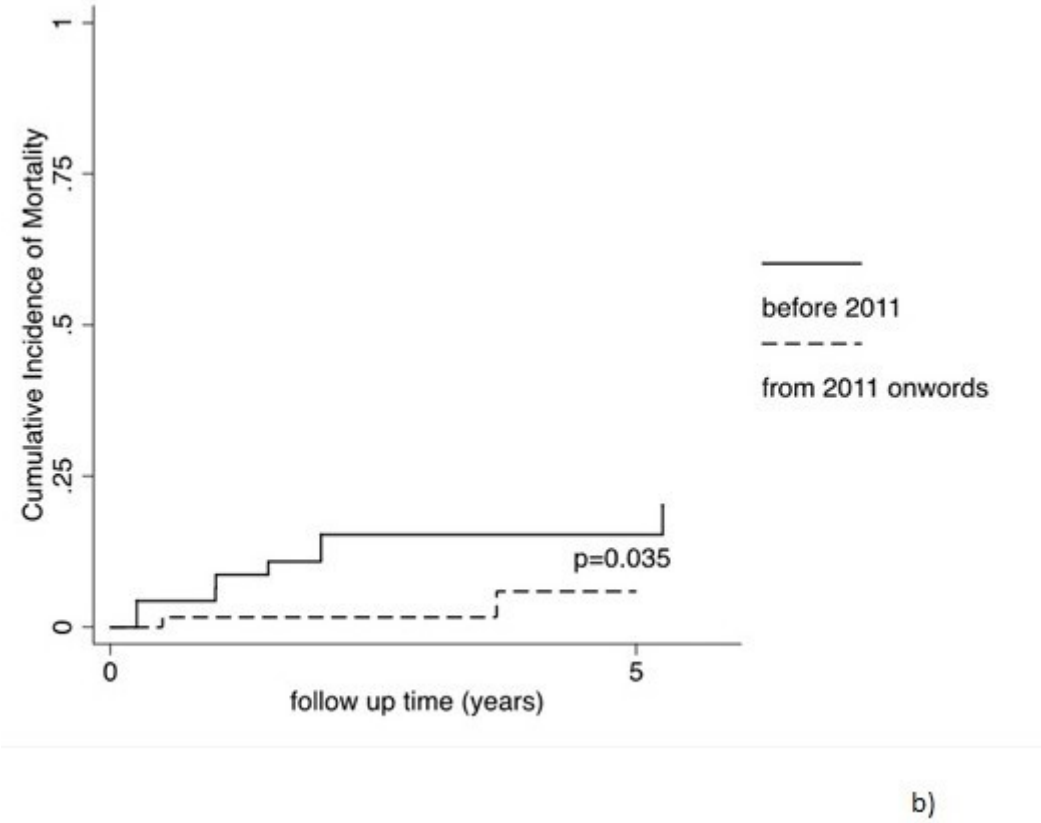
Everolimus obtained the approval with these indications by the European Medicines Agency in 2011 and by US Food and Drug Administration in 2018

Bissler JJ et al. Lancet 2013
Franz DN et al Lancet 2013
French JA Lancet 2016

Sirolimus did change the history of the disease



Individual trajectories observed over time (gray lines) of FEV1 (L), with mean slope before and after the baseline (bold dark line) estimated from s linear mixed model



Cumulative incidence of mortality before and after the use of sirolimus in our center on 105 patients

Harari S. et al Respiration 2021

All that glitters is not gold…

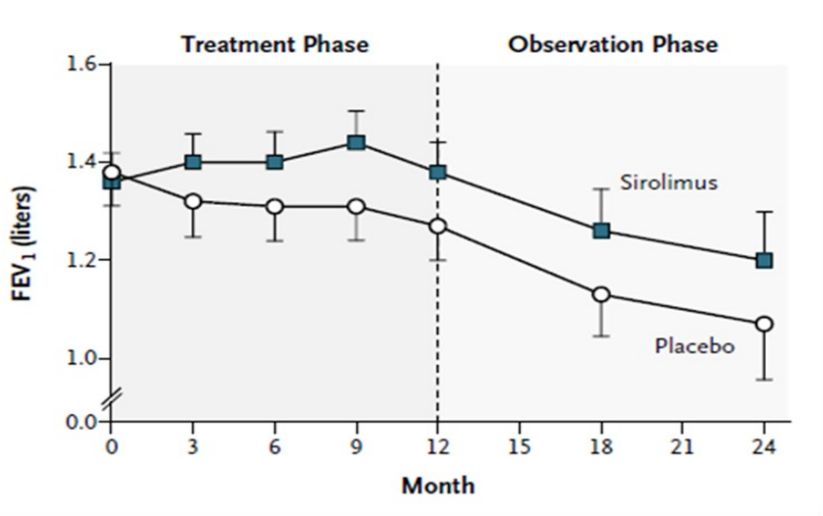
Decline of FEV1 after an initial stabilization during treatment with sirolimus can occur

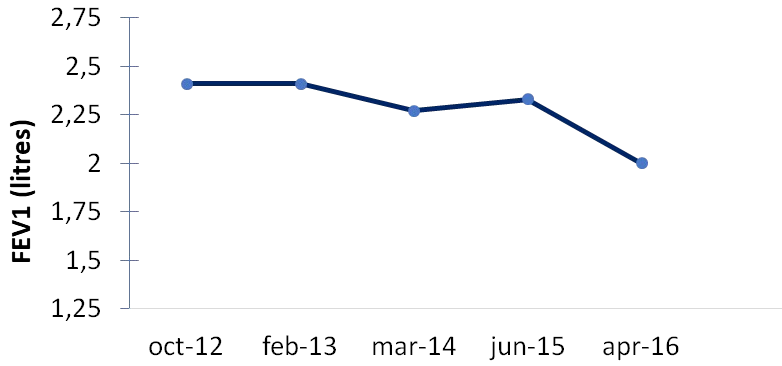
12 patients out of 33 treated for progressive disease for at least 3 years experienced an important reduction in FEV1 (≥ 90mL/year) in the last 12 months of observation (two among them after 48 months of treatment).

The mean FEV1 change over the last 12 months of observation was –0.354 mL/year

No significant differences were seen regarding the age at diagnosis, the age at treatment initiation and pulmonary function at baseline between 21 patients with stable or improved lung function and patients who experienced worsening of lung function during the last 12 months of treatment.

VEGF-D at baseline was higher in patients with stable lung function during treatment than in patients with a decline in FEV1, FVC and DLCO higher than 10%, even though the difference was not statistically significant





Harari S. et al Respiration 2021

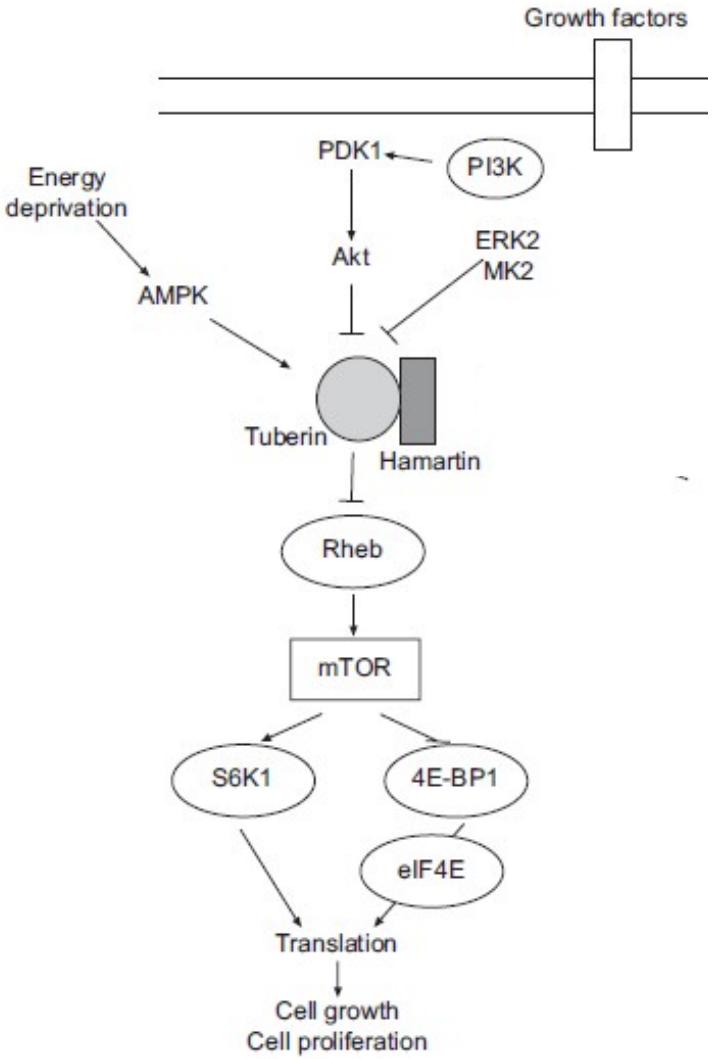
Clinical trials

Name	Drug	Design	Primary endpoint	Status	Results
MILES	Sirolimus	Randomized, d. blind, placebo controlled	FEV1	Completed	Positive results
	Everolimus	Open label, dose-escalating	Safety, PK, PD	Completed	Positive - Lower safety profile compared to sirolimus
SAIL	Sirolimus plus Hydroxychloroquine	Open label	Safety	Completed	Positive results at higher dose
TRAIL	Letrozole	Randomized, d. blind, placebo controlled	FEV1	Completed	Failed to enroll the target n of pts
	Doxycycline	Randomized, d. blind, placebo controlled	FEV1	Completed	Negative results
SLAM-2	Saracatinib	Open label, Single arm	FEV1	Completed	Result not available
COLA	Celecoxib	Open label, Single arm	Safety	Completed	Result not available
LAM	Nintedanib	Open label Single arm	FEV1	Completed	Result not available
LAMP1	Imatinib	Randomized, blind, placebo controlled	VEGFD	Completed	Result not available
RESULT	Sirolimus plus Resveratrol	Open label, Single arm	VEGFD	Completed	Negative results
SOS	mTOR inhibitors plus simvastatin	Open label Single arm	Safety	Completed	Negative - associated with decline in FEV1

A jump into the pathogenesis…

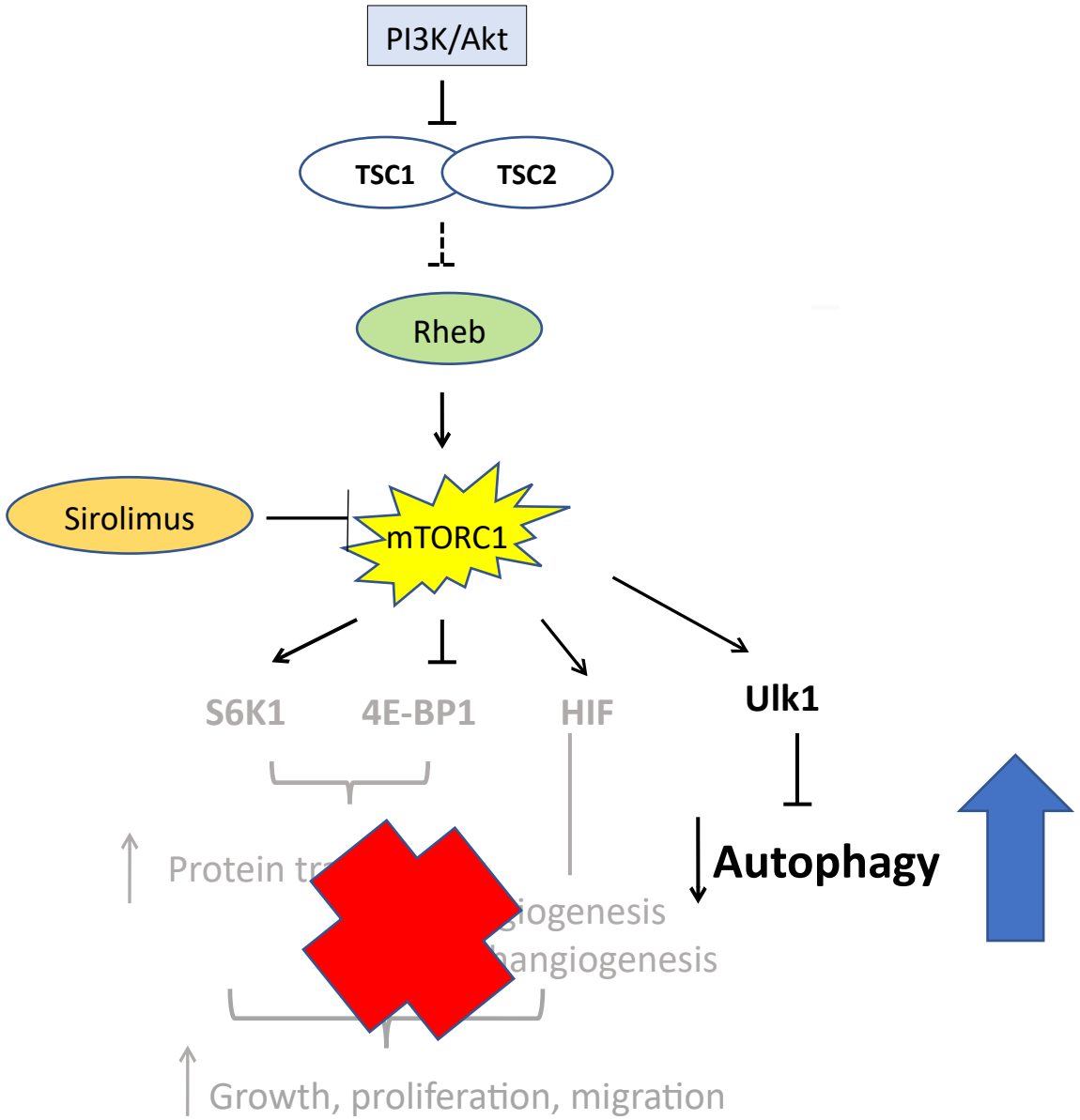
In vivo, hamartin and tuberin are part of a heterotrimer acting upstream of the mTOR, whose major function is regulation of cell growth and proliferation mediated by growth factors, energy and stress signals.

Inactivating mutations of hamartin and tuberin result in a constitutive activation of mTOR pathway leading to enhanced cell growth and proliferation



Harari et al, ERR 2011

A jump into the pathogenesis…



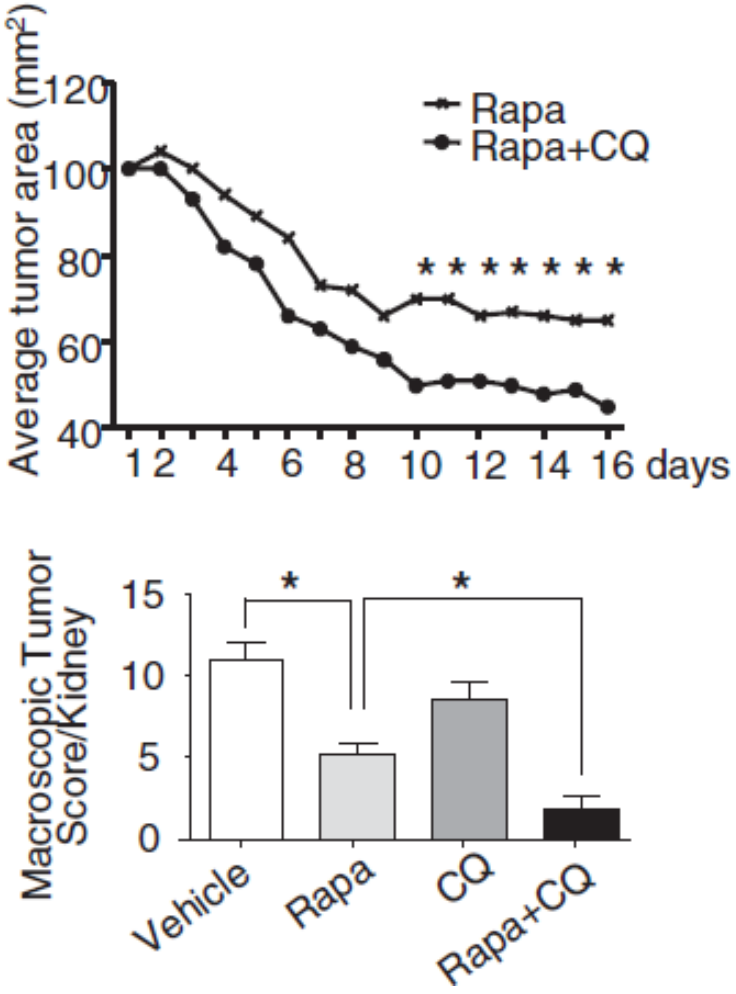
Autophagy allows cells to produce energy and maintain essential molecules through the use of breakdown products.

mTORC1 is a known inhibitor of autophagy

LAM cells are predicted to have low levels of autophagy, because of mTORC1 activation, leading to a reduced cell survival

Sirolimus blocks the further growth of LAM cells by inhibiting protein translation, but simultaneously promotes the survival of LAM cells by inducing autophagy.

Autophagy and mTORC



The combination of mTORC1 and autophagy inhibition (using rapamycin and chloroquine) is more effective than either treatment alone in inhibiting the survival of tuberin (TSC2)- null cells, growth of TSC2-null xenograft tumors, and development of spontaneous renal tumors in Tsc2+/− mice

Parkhitko A et al, PNAS 2011

Sirolimus and Autophagy Inhibition in LAM

A safety and tolerability trial of Sirolimus and hydroxychloroquine

A phase I clinical trial

Open label

24 weeks treatment phase followed by 24 weeks observation phase

3 patients received 200 mg of hydroxychloroquine and 10 patients 400 mg of hydroxychloroquine

Results:

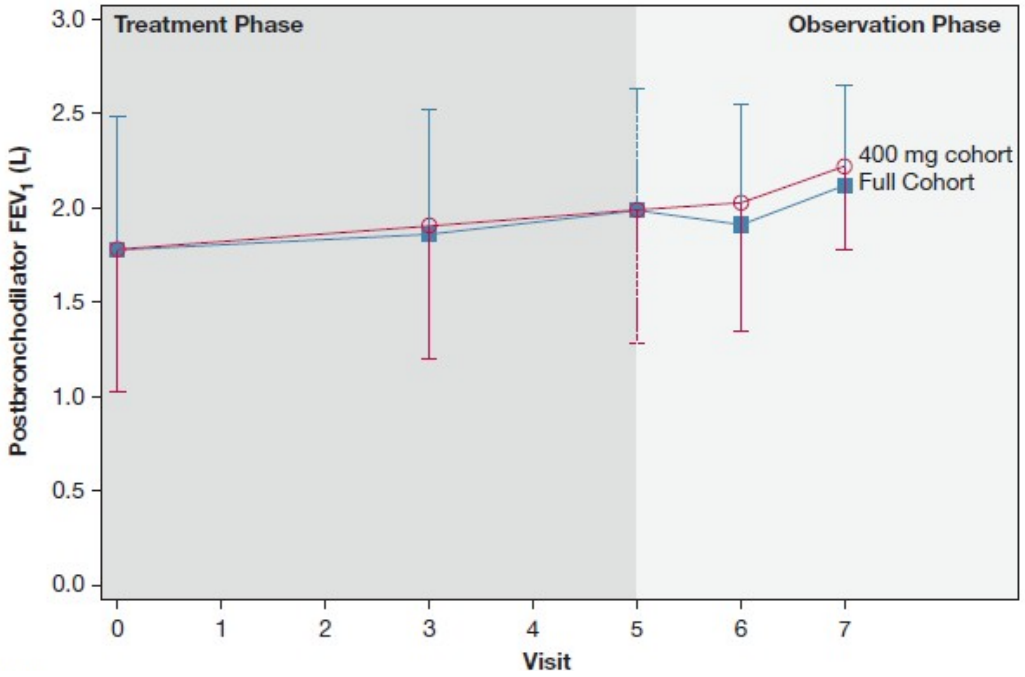
✓ Most common adverse events: mucositis, headache, diarrhoea

✓ No drug-related SAEs

✓ Improvement in lung function at 24 weeks as those observed during sirolimus therapy, with a decrease in lung function at 48-weeks.

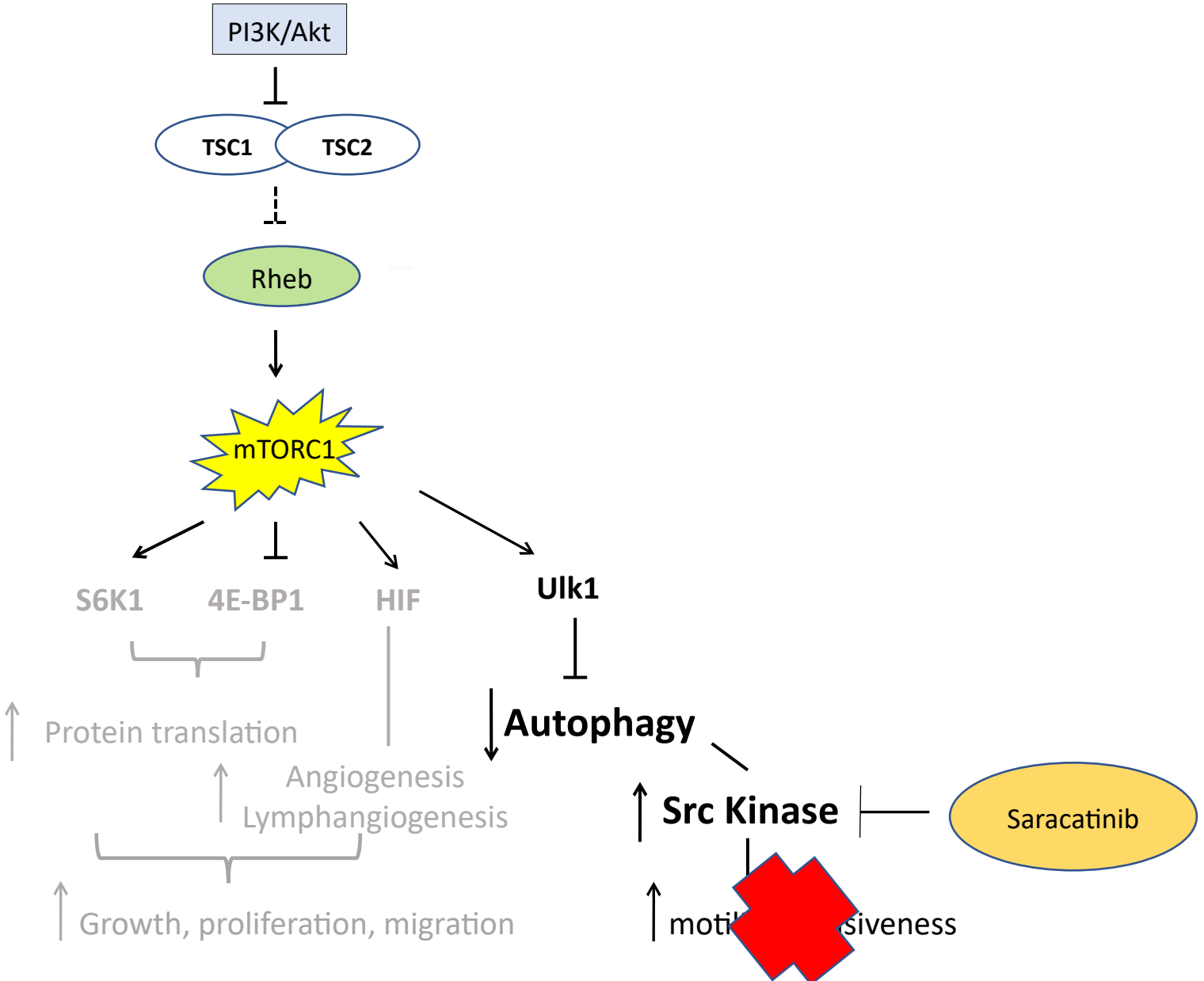
When the higher dose of Hydroxychloroquine was analyzed separately, FEV1 and FVC remained Stable at 48 weeks (end of observation)

No change in SGRQ



El-Chemaly S et al, Chest 2017

A jump into the pathogenesis…



Src inhibition in LAM

"SLAM-1"

phase 1, open label, single arm

Estimated enrolment 12 patients

Safety of three escalating doses of saracatinib, 50-125-175mg die

"SLAM-2"

Safety and efficacy of 125m

phase 2, open label, single a

Duration of treatment: 9 months

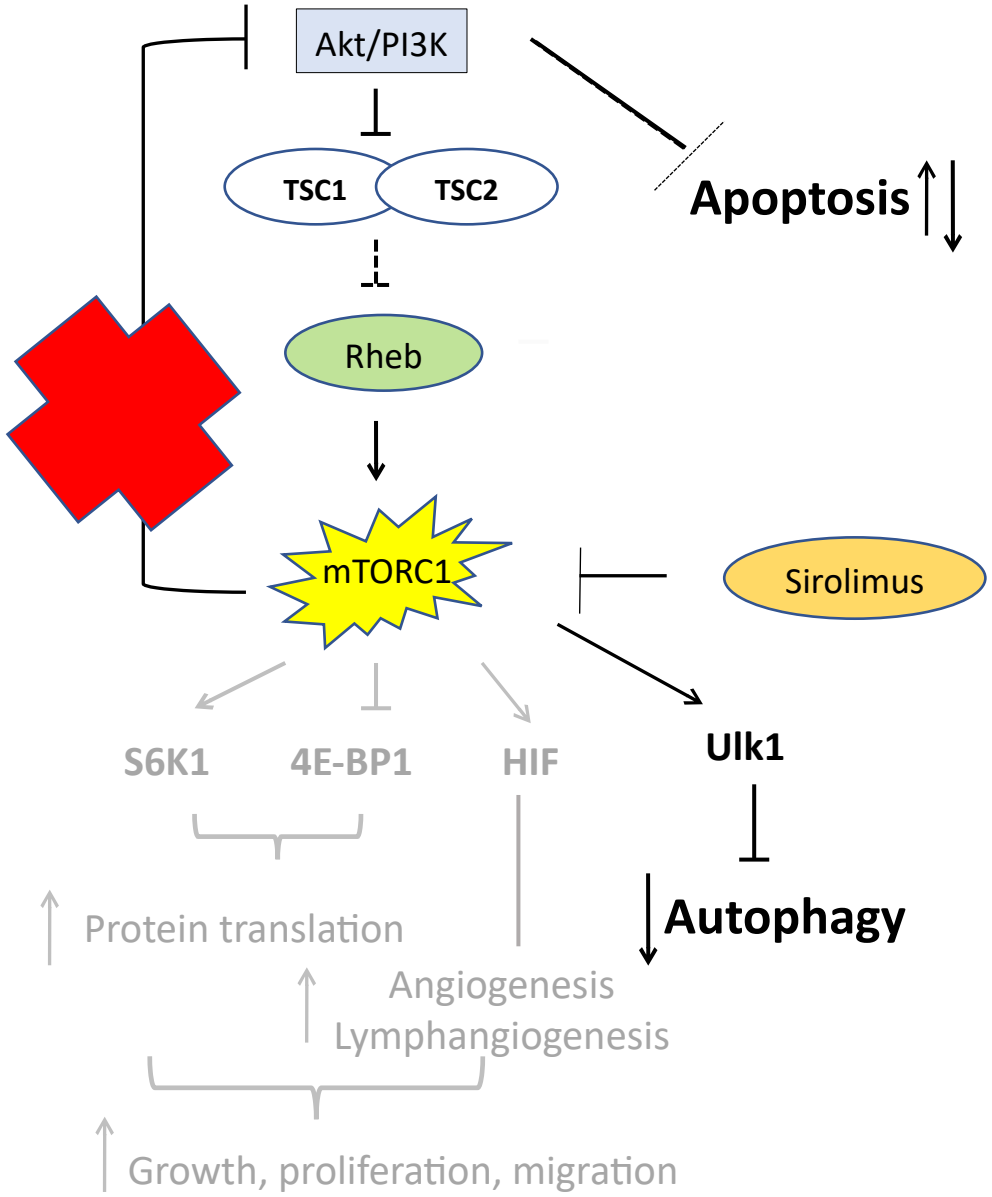
Both completed
results not still published

Inclusion criteria: a recent reduction in forced expiratory volume at 1-second (FEV1) of > 50ml/year

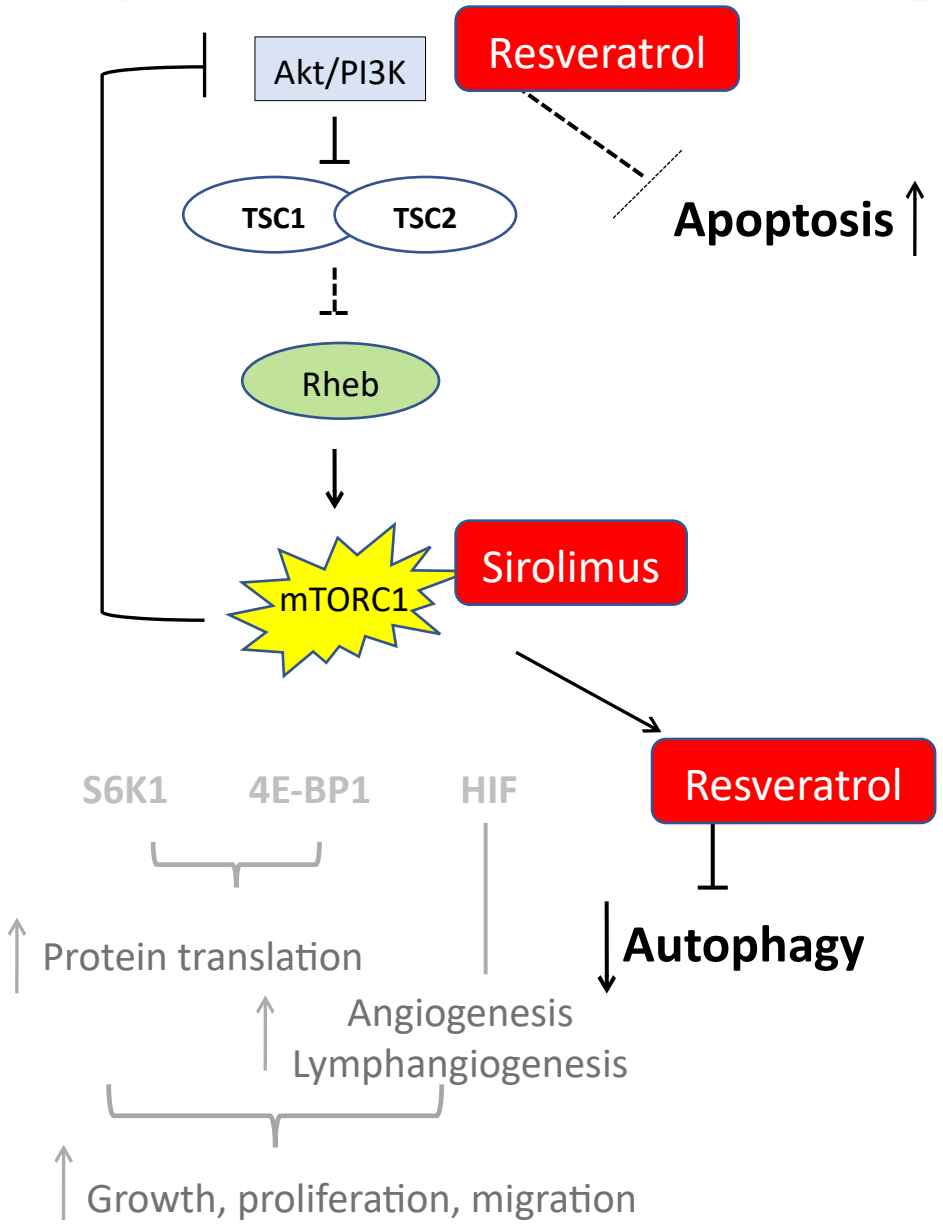
28 patients enrolled

- Primary Outcome Measures: FEV1
- Secondary Outcome Measures: AML measured volumetrically on MRI
  Lung Cyst size measured on chest CT
  serum VEGF-D levels

A jump into the pathogenesis…



A jump into the pathogenesis…

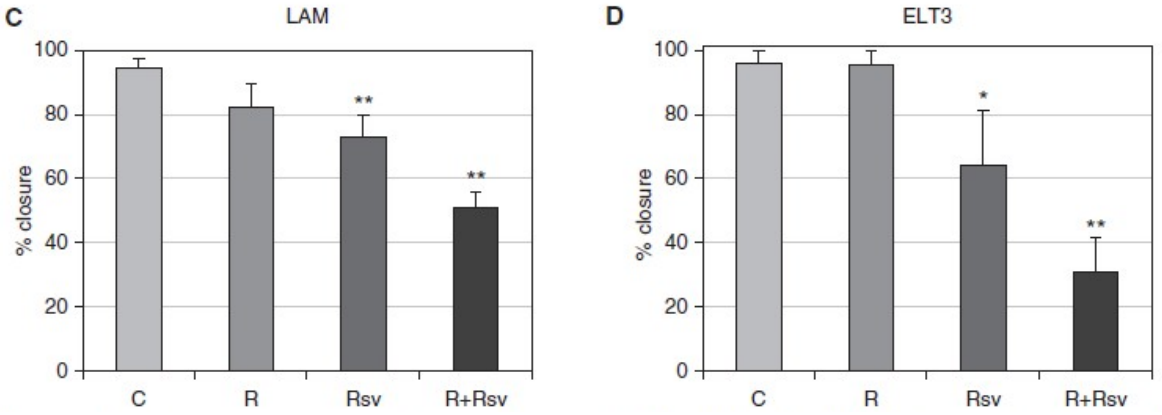


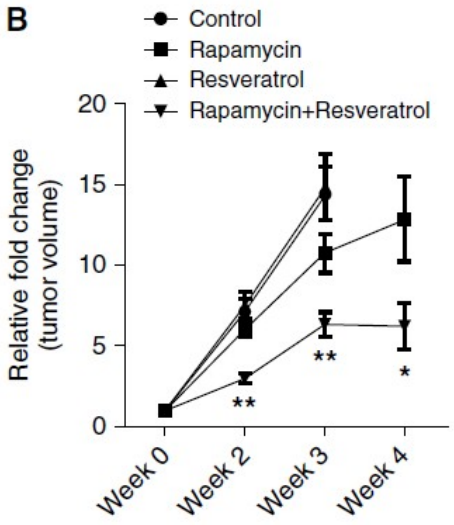
Resveratrol is a naturally occurring polyphenol that is found in red wine, grapes, and peanuts and possesses disease-protective and antiaging properties.

Resveratrol suppress proliferation of human cells in vitro and mediate anti-tumor effects through several signaling pathways by modulating cellular events associated with autophagy, cell growth, and proliferation

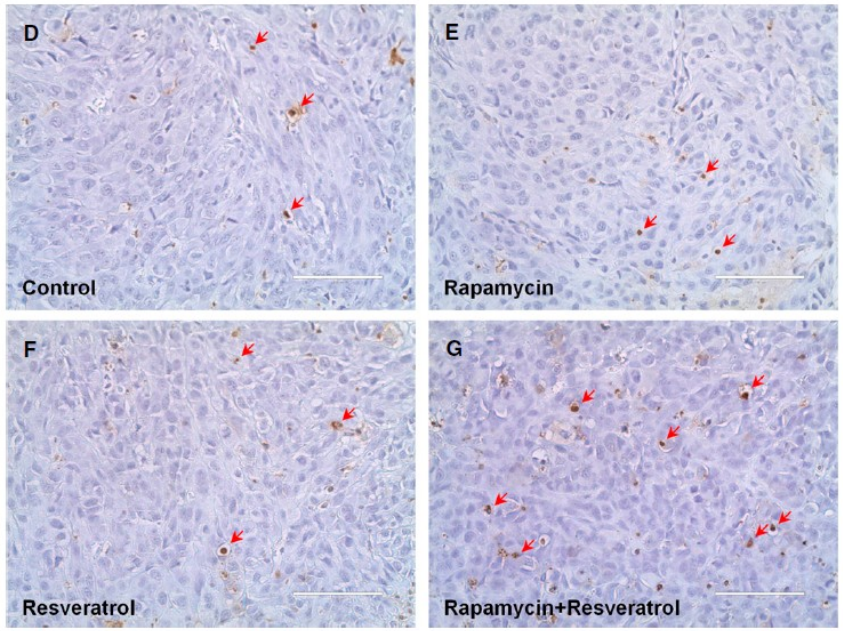
Combination of rapamycin and resveratrol

Rapamycin + resveratrol reduce cell migration of LAM and ELT3 cells





Rapamycin + resveratrol reduce xenograft tumor size



Rapamycin + resveratrol induce apoptosis in xenograft tumors.

Alayev A et al, Am J Respir Cell Mol Biol 2015

Resveratrol and Sirolimus in LAM

Safety and efficacy of escalating dose (250, 500, 1000 mg) of resveratrol in combination with sirolimus

phase 2, open label, single arm,

Duration of treatment: 24 weeks

Inclusion criteria: pts with stable disease with sirolimus for at least 20 weeks

Study population: 25 LAM patients (51 years, range 47-58)

- Primary Outcome Measures: serum VEGFD levels
- Secondary Outcome Measures: safety
  lung function (FEV1,FVC, DLCO)
  quality of life

Gupta N et al. Chest feb 2023 *in press*

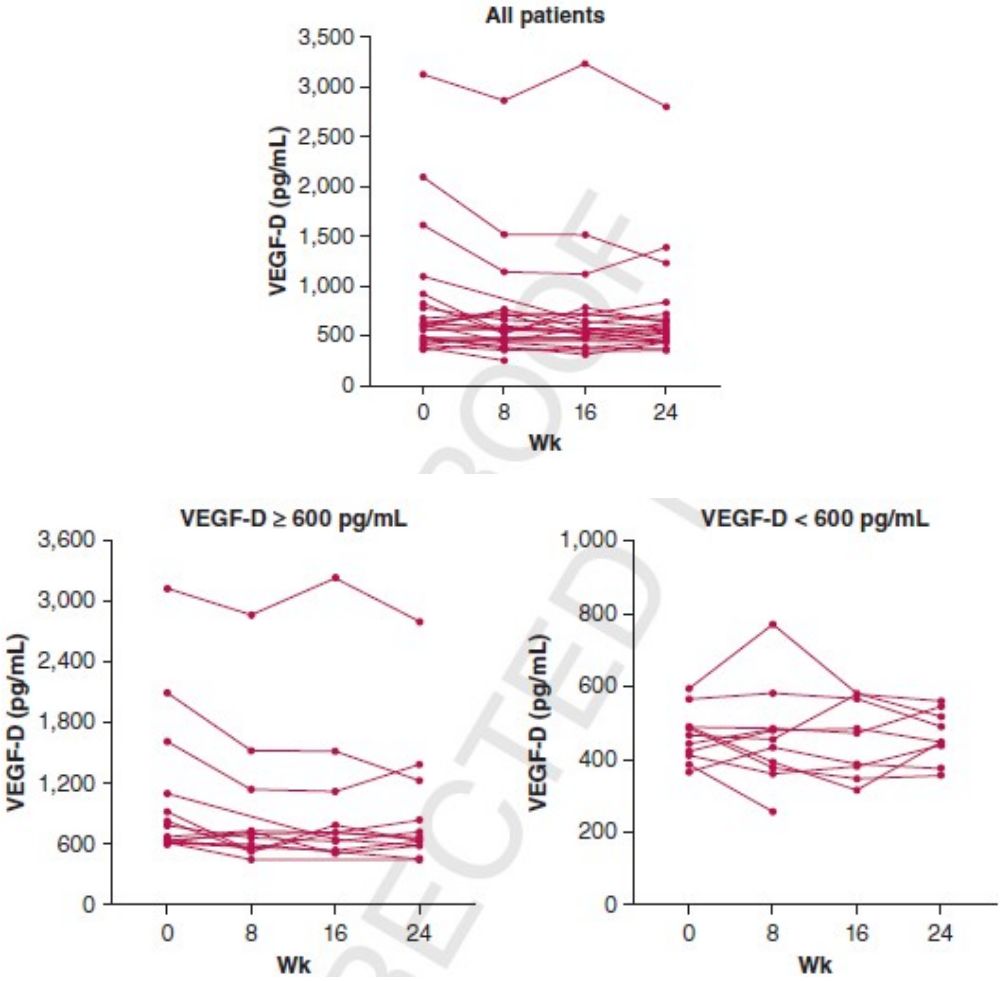
Resveratrol and Sirolimus in LAM: results

The prespecified primary outcome of ≥42% reduction in serum VEGF-D levels after 24 weeks of combined sirolimus and resveratrol was not reached, however a statistically significant reduction in VEGF-D levels was observed during the study (802 pg/mL at baseline to 721 pg/mL at 24 week)

Despite some GI side effects, the addition of resveratrol was well tolerated.

Patients showed moderate to severe physiologic impairment at baseline. During the 24-week study duration, models showed a modest but statistically significant reduction in FEV1, but no significant changes in FVC or DLCO

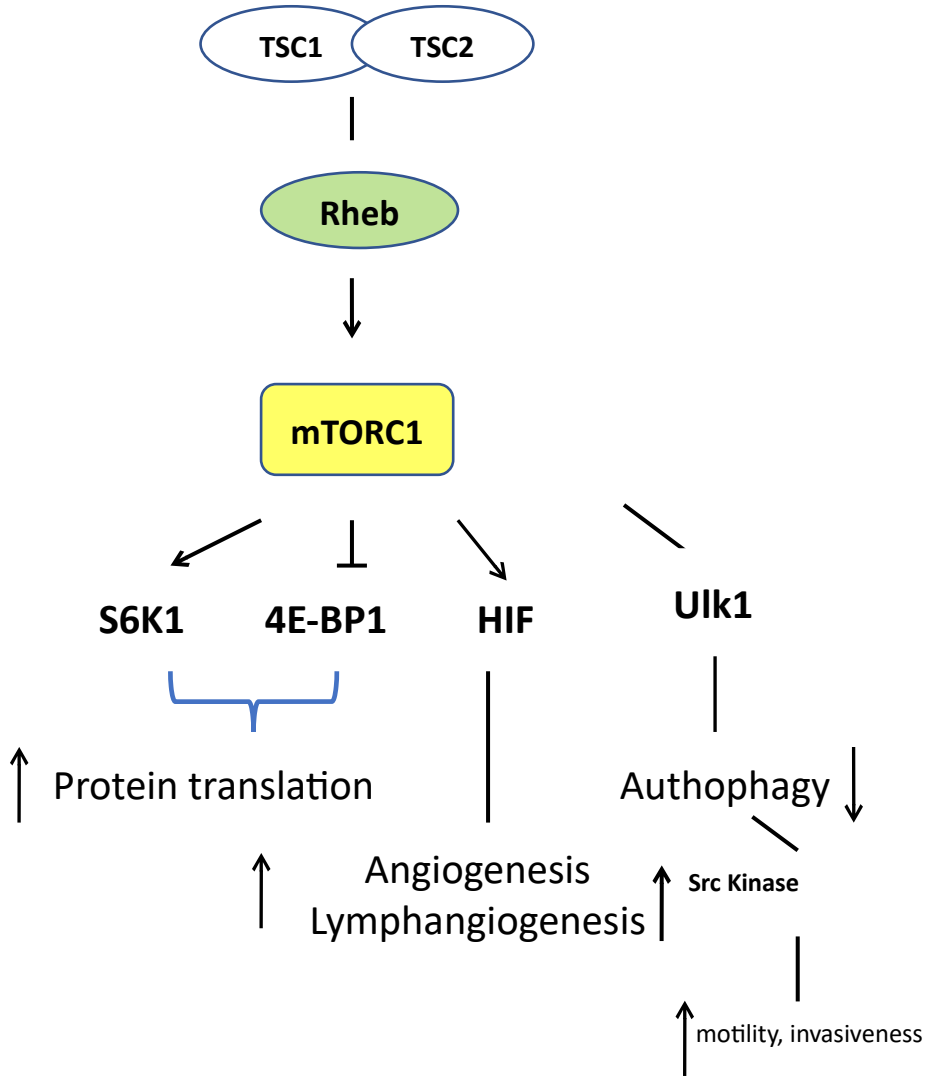
Modest improvement in HRQOL was found



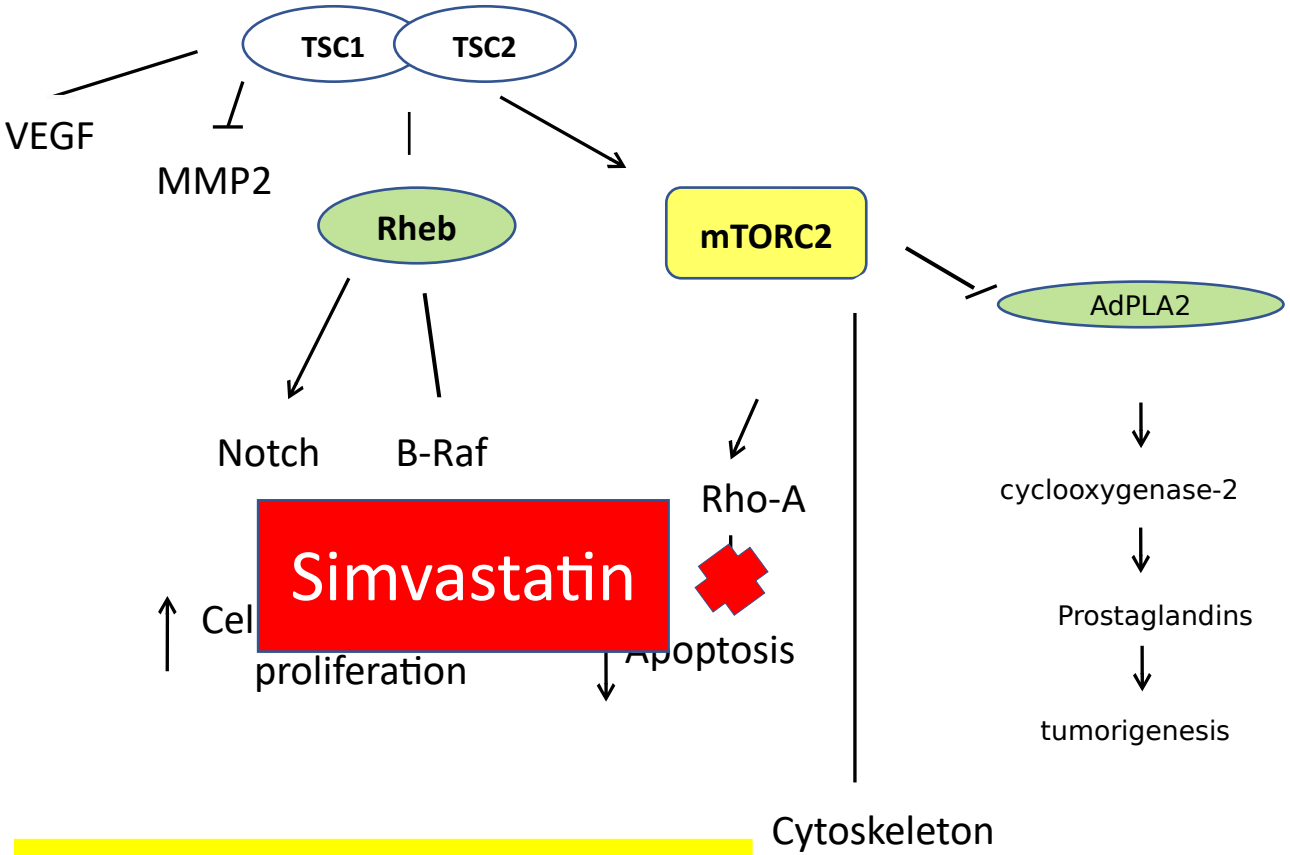
Gupta N et al. Chest feb 2023 *in press*

Not only canonical TSC pathway

Canonical TSC pathway



Non canonical TSC pathways



Simvastatin inhibited RhoA activity and promoted apoptosis in vivo and animal models

mTOR Inhibition and statins in LAM

"SOS"

Safety of

phase 1-2,

- Primary C

- Secondar

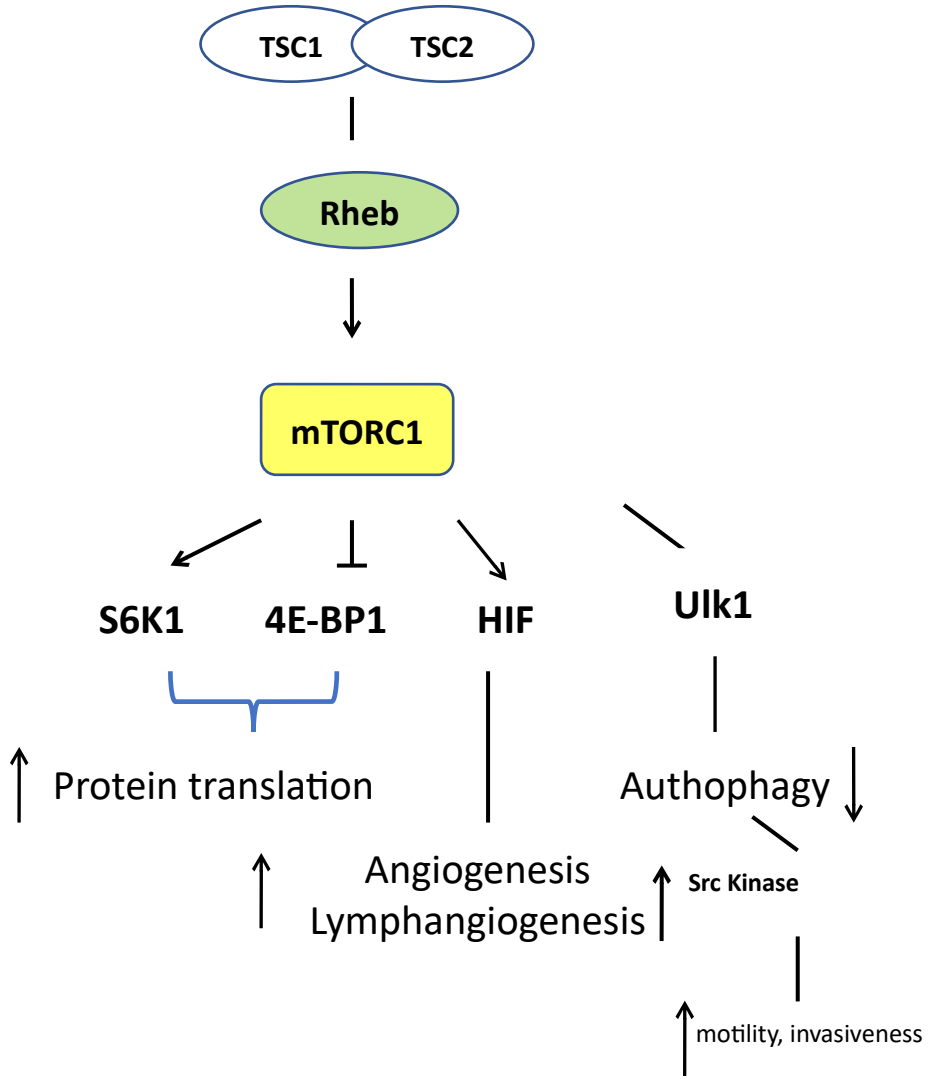
10 pts were treated with combination therapy >3 months with 20 mg simvastatin for two months followed by 40 mg for two months. The most common adverse events were peripheral edema (30%), cough (30%), and diarrhea (30%). No patients withdrew or had a reduction in simvastatin dose because of adverse events.

The combination of simvastatin with mTORi in LAM patients is safe and well-tolerated from an adverse events perspective. The addition of simvastatin, however, was associated with decline in FEV1.

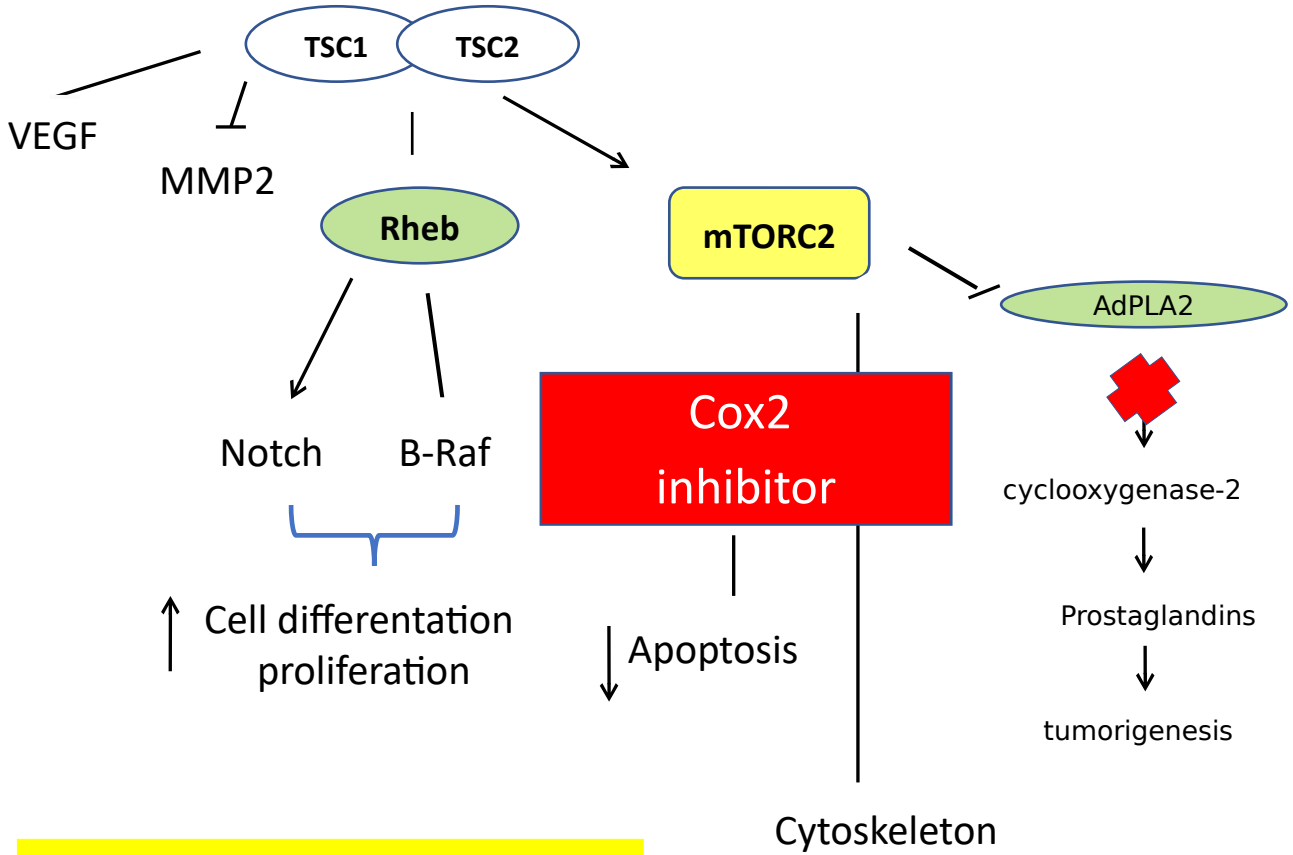
Krymskaya VP et coll. Respir Med 2020

Not only canonical TSC pathway

Canonical TSC pathway



Non canonical TSC pathways



Secreted levels of PGs are higher from TSC2 deficient cells than TSC-addback cells and are not affected by rapamycin

COX-2 Inhibition in LAM

A Pilot Clinical Trial of COX-2 Inhibition (celecoxib) in sporadic LAM and TSC
phase 1, open label, single arm

Duration of study: 6 months treatment period, 6 months follow-up

Inclusion criteria: no mTOR inhibitors, mild disease

- Primary Outcome Measures: safety and tolerability

- Secondary Outcome Measures: lung function (FEV1)
  AML size (MRI)
  serum VEGFD levels
  Exhaled breath condensate PGs
  Quality of life

El-Chemaly S ERJ 2020

COX-2 Inhibition in LAM

From June 2016 through November 2018, 12 were enrolled on the study drug.

No dose-limiting toxicities were encountered.

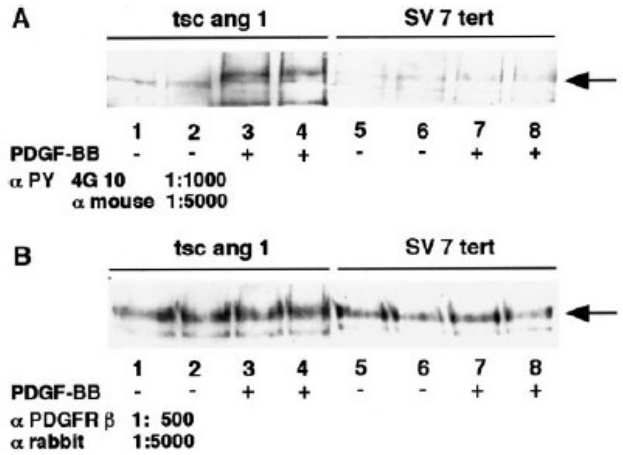
A total of 75 non-serious adverse events were reported for 11 patients. Nearly half (37/75) were thought to be related to celecoxib.

The most commonly reported non-serious adverse events were stomach/abdominal pain (six events from three subjects) and headaches (six events from three subjects).

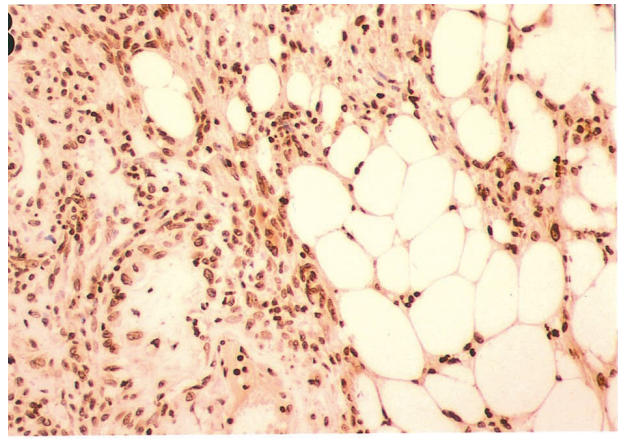
The least square mean of post-bronchodilator forced vital capacity (FVC) (mL), post-bronchodilator FEV1 and diffusing capacity of the lung for carbon monoxide (DLCO) did not change significantly during the study period

El-Chemaly S ERJ 2020

Nintedanib in LAM



Human AML derived cell line and murine TSC2 mutated cells express functional PDGFR



Markedly positive staining of human angiomyolipoma with phosphospecific PDGFR

Arbiser JL et al, Am J Pathol 2002

In TSC2 deficient cells, growth can be enhanced due to a oestrogen-induced activation of a PDGFRb and ERK1/2 signalling pathway

Finlay GA et al, J Biol Chem 2004

The simultaneous inhibition of VEGF, PDGF and FGF signaling pathways reduces tumor angiogenesis in lung.

As angiogenesis and lymphangiogenesis are crucial mechanisms involved in dissemination of LAM cells, potential inhibition by nintedanib may contribute to prevent disease progression in LAM

Nintedanib in LAM

A pilot study of efficacy and safety of nintedanib in LAM phase 2, open label, single arm, IIS

Study duration: 12 months of treatment followed by 12 months of observation

Study drug: Ninedanib 150 mg bid. Drug dosage can been reduced to 100 mg bid in case of liver toxicity/SE

Inclusion criteria: evidence of progression of disease (≥80ml/year or 10% FEV1)

or intolerance/toxicities to sirolimus

Nintedanib in LAM: end points

Primary end point:

✓FEV1 improvement

Secondary end points:

✓Safety and Tolerability

✓The proportion of patients achieving a stabilization of the lung function during treatment. A patient will be considered stabilized if her value of the FEV1 measured at 12-month visit will be equal or above the baseline value.

✓Rate of decline of FVC and DLCO over the course of the study

✓VEGF-D levels: change from baseline at the end of treatment period

✓Renal angiomyolipoma size (MRI diameter > 1 < 5 cm).

✓Circulating LAM cells

✓Quality of life St. George and EQ5d (change from baseline)

Take home messages

1. To date mTOR inhibitors are the only proven treatment for LAM

2. Recent advances in the understanding of the pathogenesis of LAM are having significant therapeutic implications

3. Several treatments have been investigated in phase 1 and 2 trials as a single agent or in combination with sirolimus, but results are not still available