LAM: beyond the cysts

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Lymphangioleiomyomatosis

Lymphangioleiomyomatosis (LAM) is a rare multisystem disease affecting almost exclusively women, occurring in sporadic form or in women with tuberous sclerosis complex

It is characterized by

- lung cysts
- fluid-filled cystic structures in the axial lymphatics
- angiomyolipomas

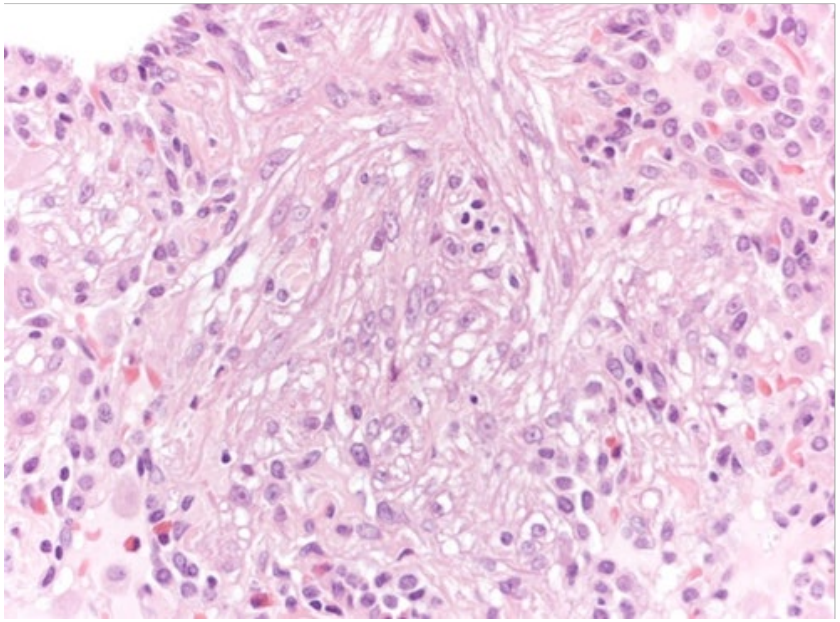
Treatment

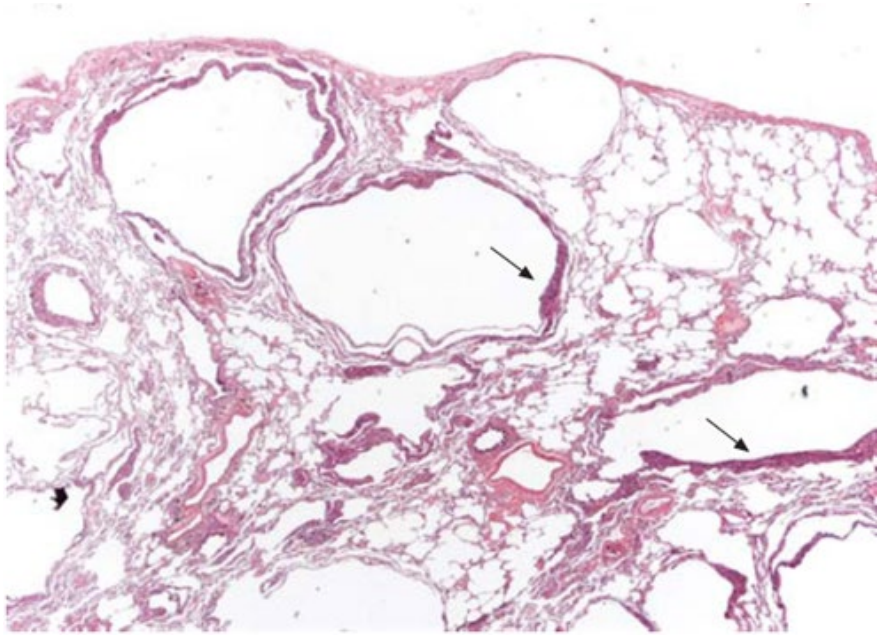
- Current therapy
- New therapeutic perspectives


pathogenesis…
clinical manifestations
…treatment

LAM - Pathogenesis

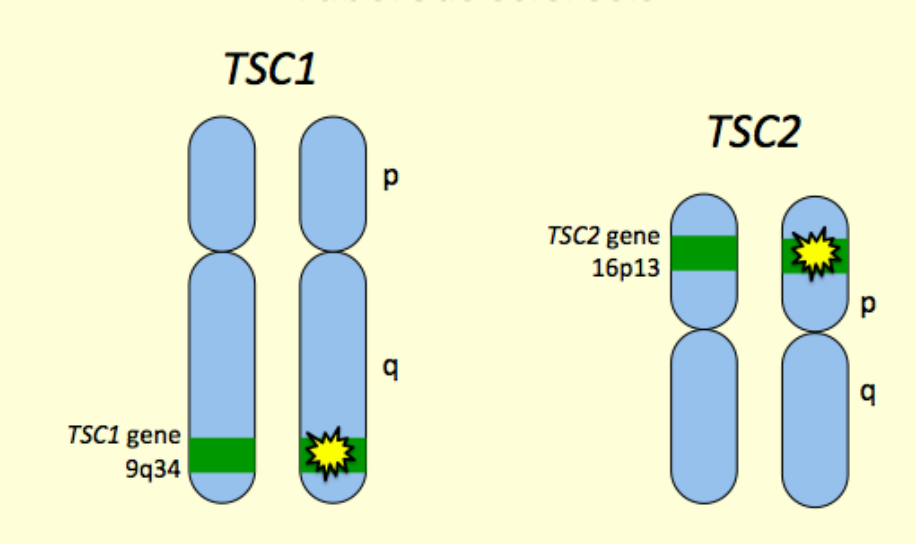
LAM is characterized by the abnormal proliferation of LAM cells expressing smooth muscle-specific proteins (such as α-actin, vimentin, and desmin), melanocyte-lineage markers (gp100 and MelanA/Mart1), and estrogen and progesterone receptors.





LAM - Etiopathogenesis

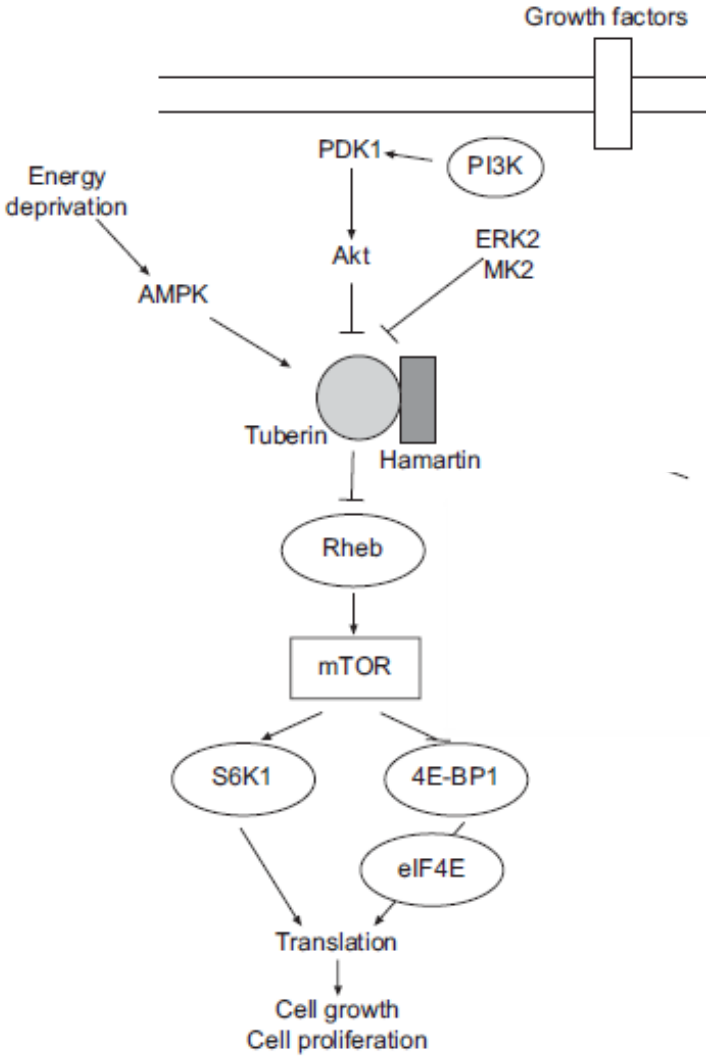
LAM occurs from mutations in the tuberous sclerosis genes TSC1 and, much more frequently, TSC2 causing the loss of function of either TSC1 or TSC2 gene products, which are known as hamartin and tuberin, respectively.



LAM - 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.

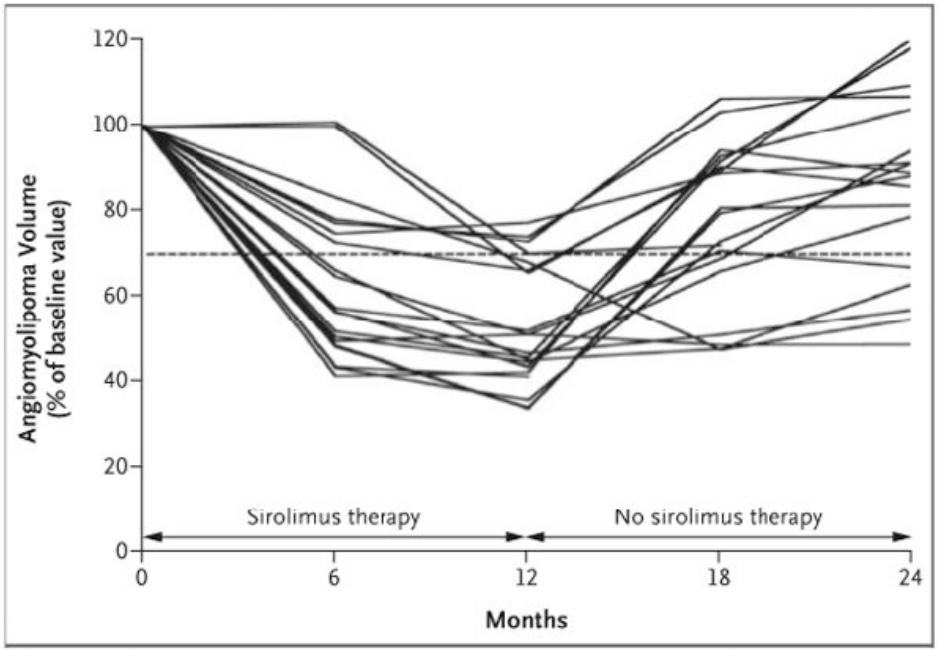
Inactivating mutations of hamartin and tuberin result in a constitutive activation of mTOR pathway leading to enhanced cell growth and proliferation

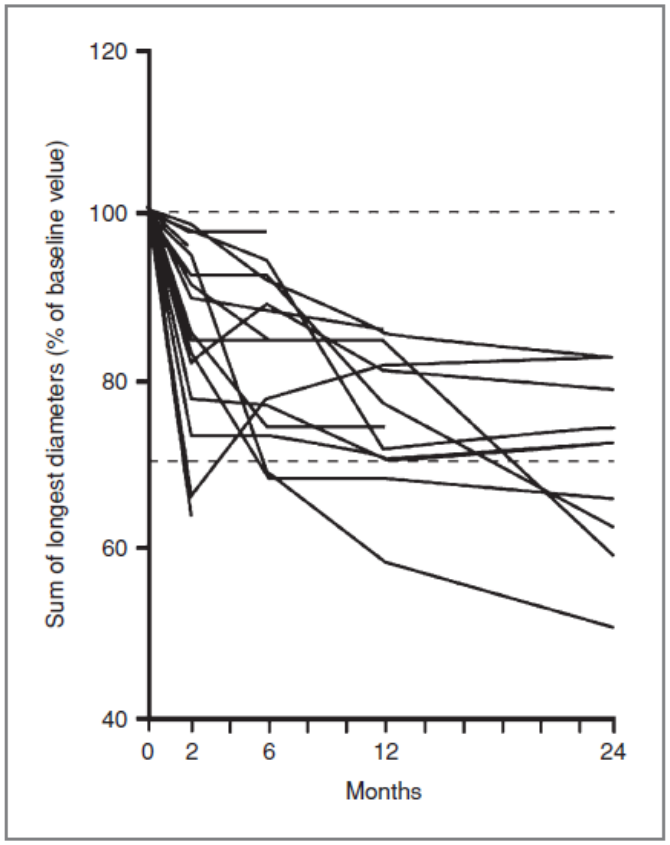


Harari et al, ERR 2011

mTOR inhibitors - sirolimus

❖Reduction in AMLs volume



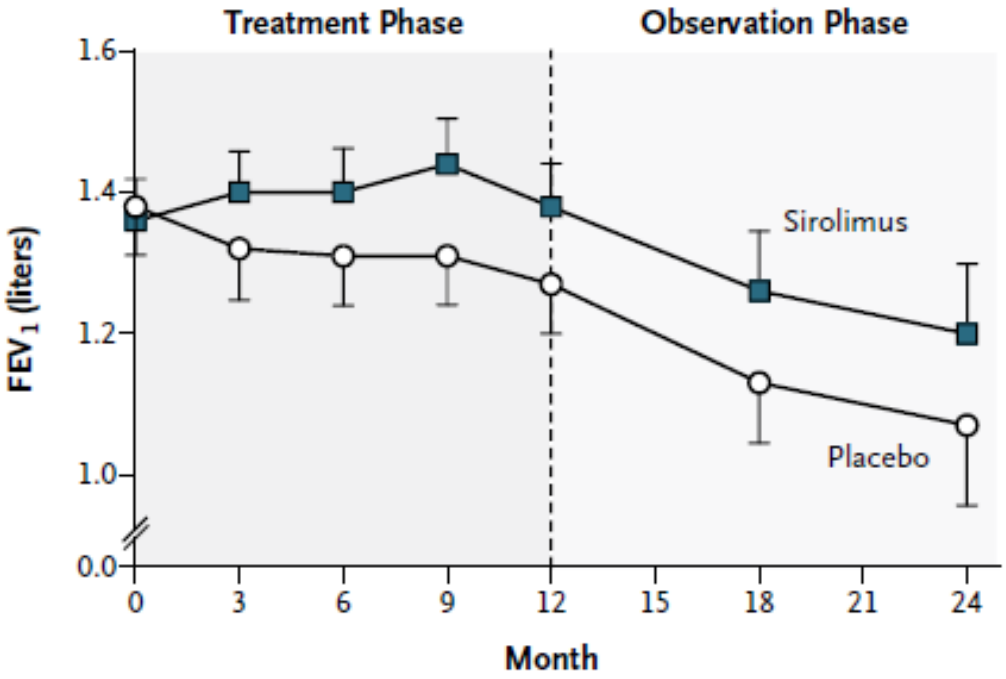


Bissler JJ et al, NEJM 2008

Davies DM, Clin Cancer Res 2011

mTOR inhibitors - sirolimus

- Stabilization of lung function (FEV1) during the treatment period

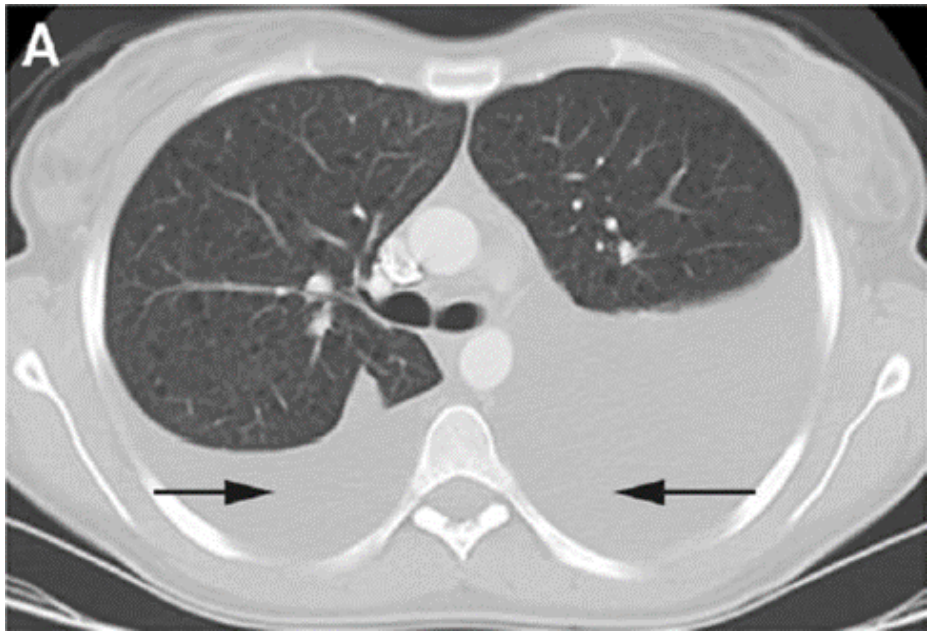


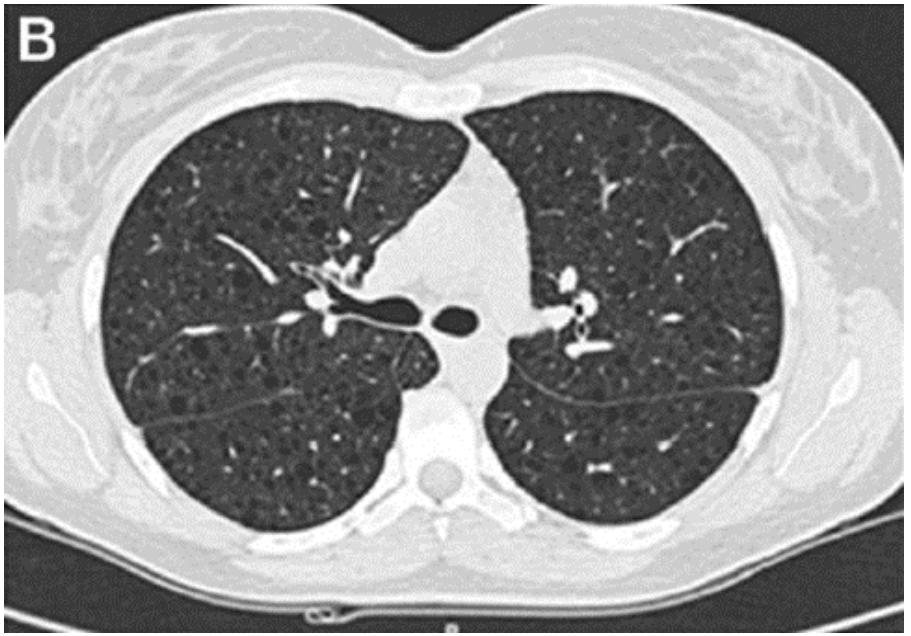
McCormack FX et al, NEJM 2011

mTOR inhibitors - sirolimus

- Resolution of chylous effusions

  Reduction of lymphangioleiomiomas





Taveira-Dasilva AM, Ann Intern Med. 2011

Sirolimus: current indications

ATS/JRS Guidelines

- Patients with abnormal lung function

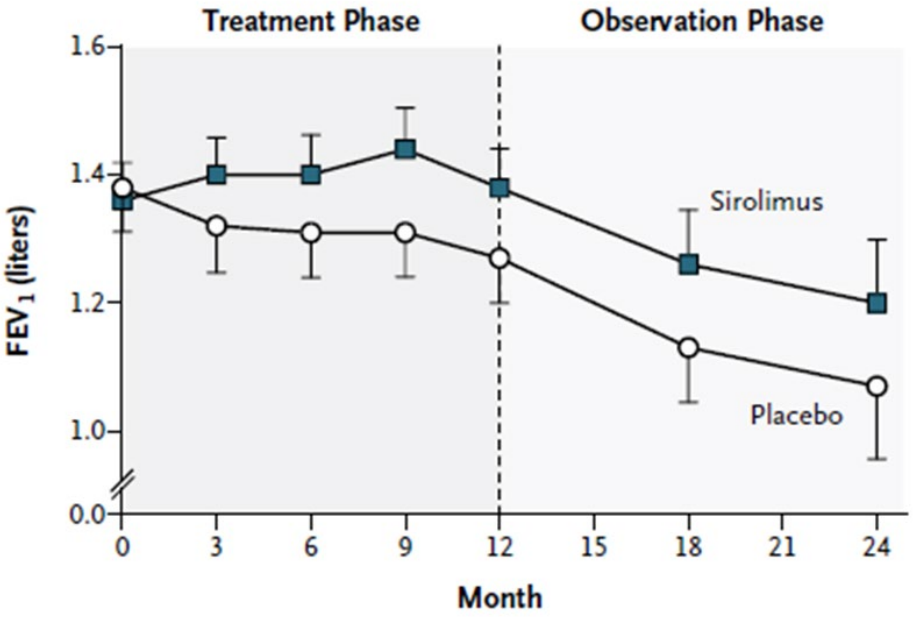
Abnormal lung function: FEV1 less than 70% predicted

- Patients whose funtion is declining

- Problematic chylous effusions and lymphangioleiomiomas

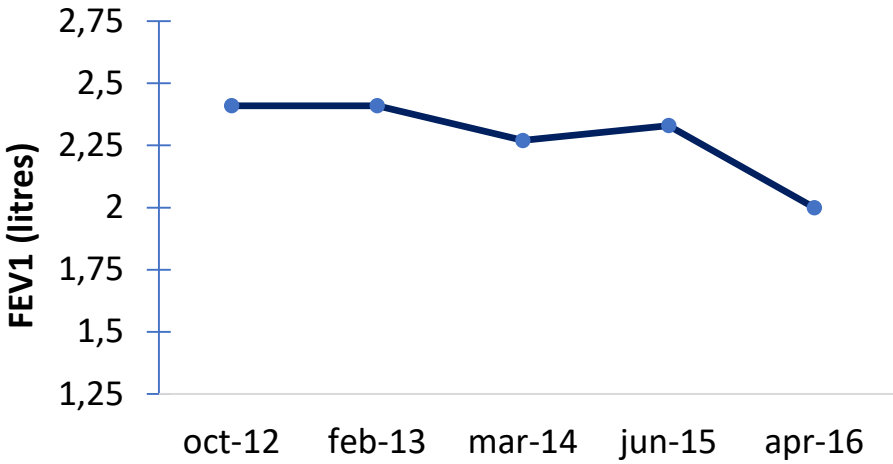
McCormack FX et al, AJRCCM 2016

Sirolimus - limits



After discontinuation of sirolimus, the decline in lung function resumed and paralleled that in the placebo group

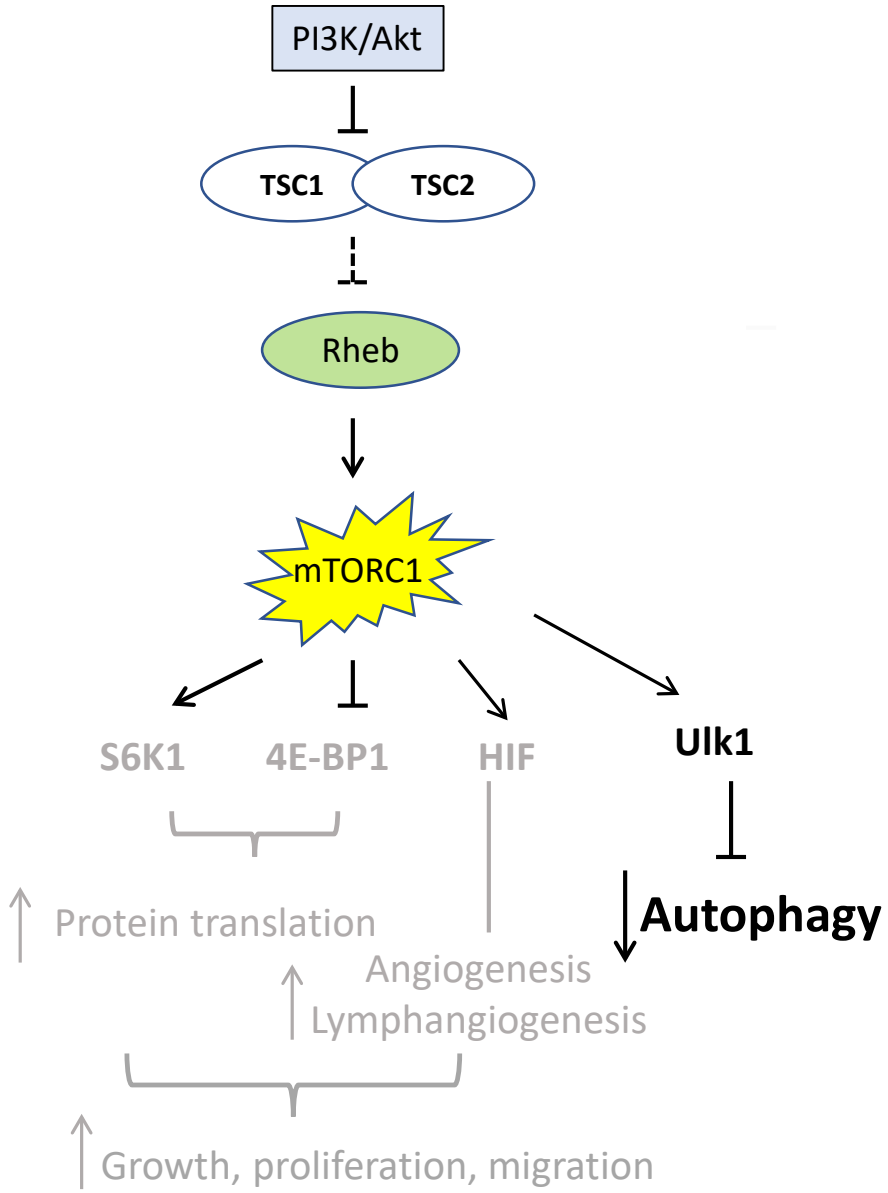
McCormack FX et al, NEJM 2011



Decline of FEV1 after an initial stabilization during treatment with sirolimus

Personal observation, unpub.

LAM - Pathogenesis



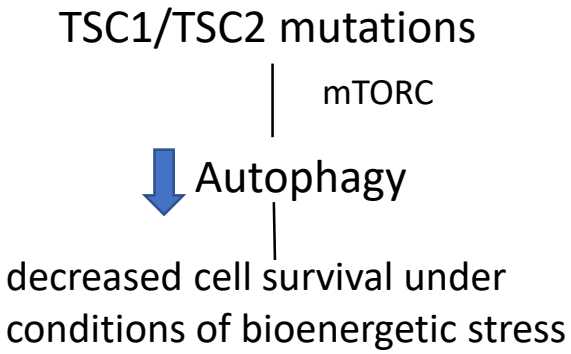
Autophagy allows cells to produce energy and maintain essential molecules through the use of breakdown products.

mTORC1 is a known inhibitor of autophagy

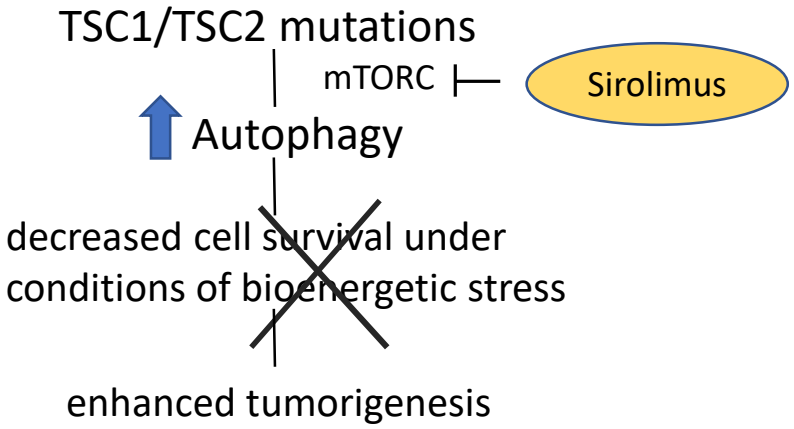
LAM cells are predicted to have low levels of autophagy, because of mTORC1 activation.

Autophagy and mTORC

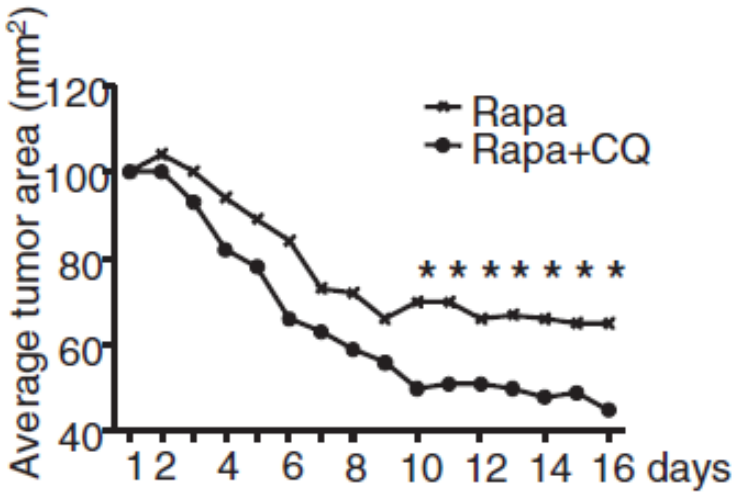
In LAM decreased autophagy can reduce cell survival

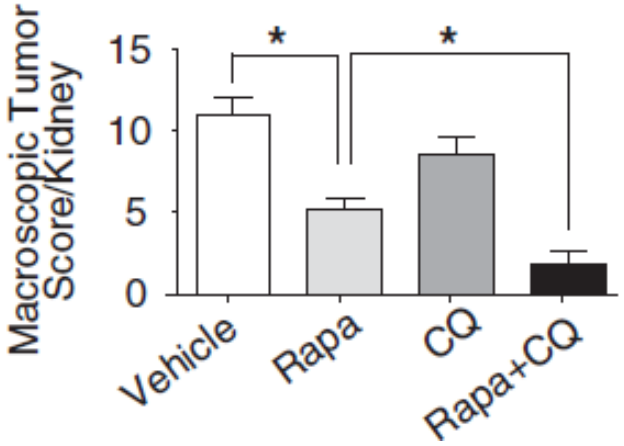


Sirolimus blocks the further growth of LAM cells by inhibiting protein translation, but simultaneously promotes the survival of LAM cells by inducing autophagy.



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 phase I clinical trial

A safety and tolerability trial of Sirolimus and hydroxychloroquine (200 and 400mg)

Open label

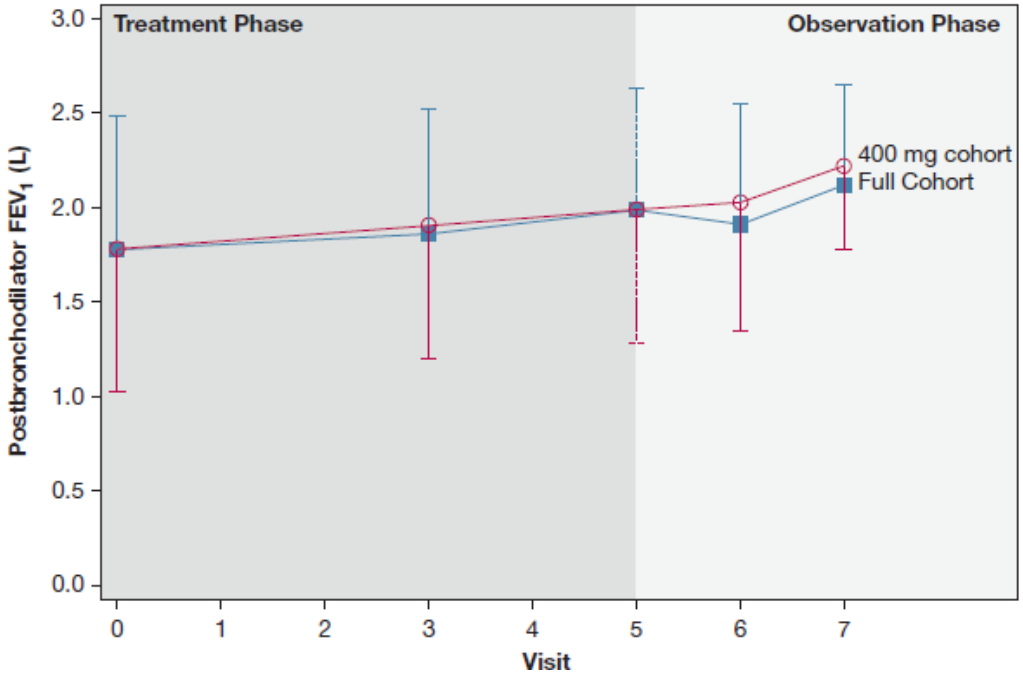
24 weeks treatment phase followed by 24 weeks observation phase

Results:

✓ Most common adverse events: mucositis, headache, diarrhea

✓ No drug-related SAEs

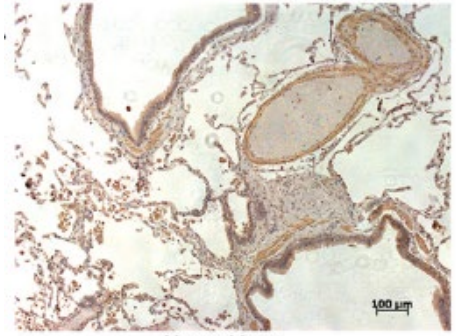
✓ Improvement in lung function at 24 weeks, with a decrease in lung function at 48-weeks. When the higher dose of Hydroxychloroquine was analyzed separately, FEV1 and FVC remained Stable at 48 weeks (end of observation)

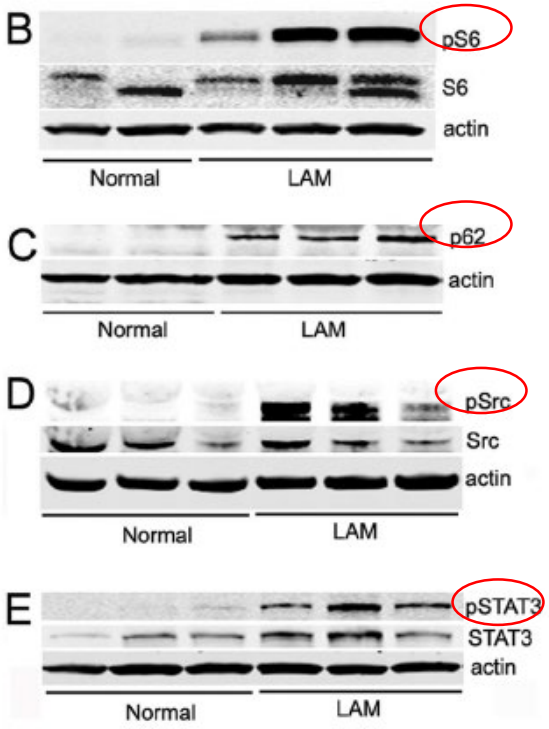


El-Chemaly S et al, Chest 2017

Src Kinase in LAM

Lung tissue



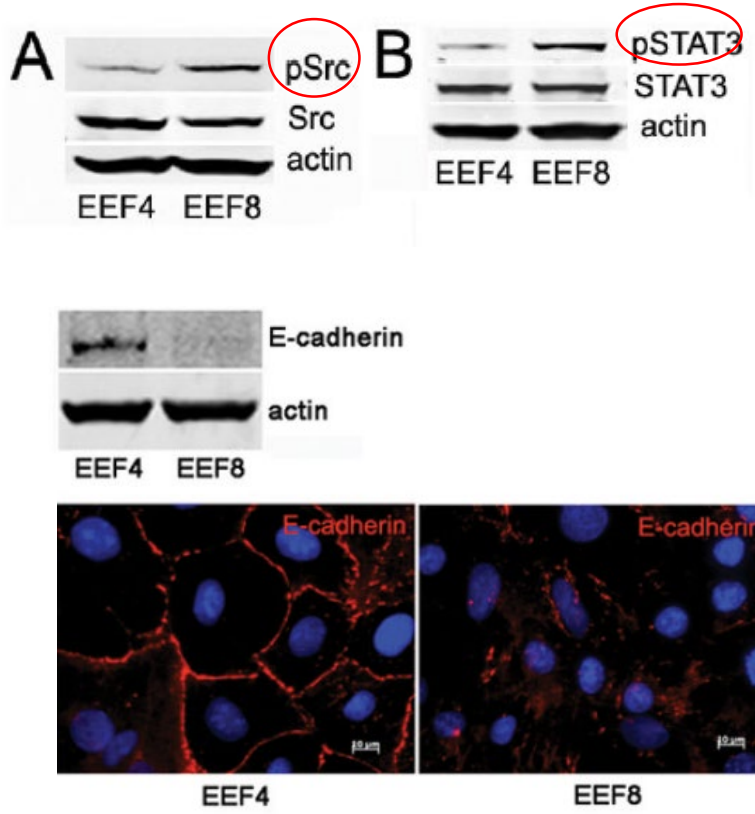


Staining with HMB45 in LAM nodules

Accumulation of
- pS6->activated mTOR
- P62->inhibited autophagy
- pSrc-> activation of Src
- pSTAT3->activated Src pathway

Eker rat embryos fibroblasts (EEF) cells

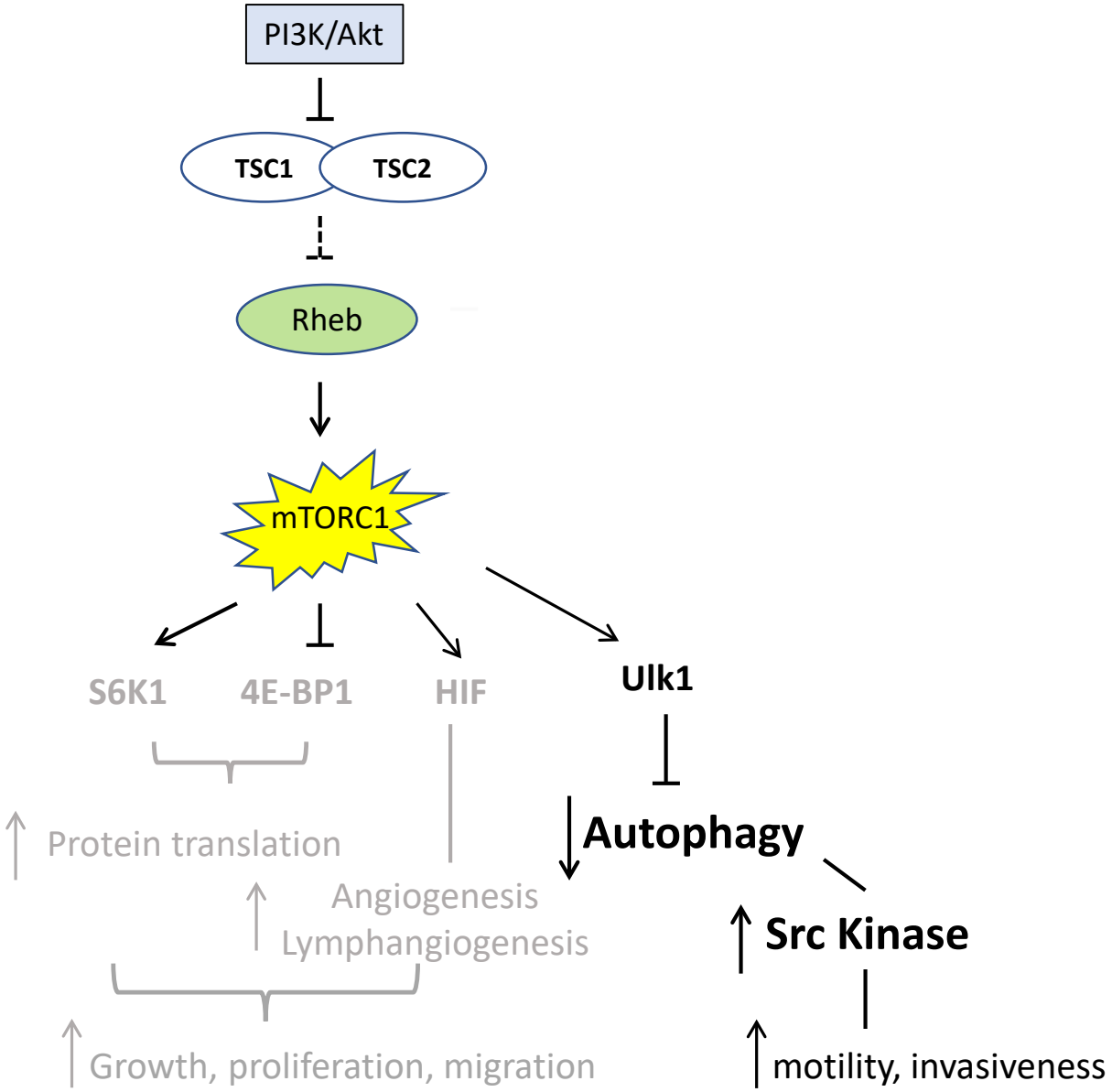
EEF4: TSC+/+
EEF8: TSC-/-



changes in the expression and localization of E-cadherin could account for the decrease in cell adhesion, increased motility, invasiveness and metastatic potential

Tyryshkin A et al, Cancer Res 2014

LAM - Pathogenesis



Src inhibition in LAM

Completed

"SLAM-1"

phase 1, open label, single arm

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

Ongoing

"SLAM-2"

Safety and efficacy of 125mg of saracatinib in LAM

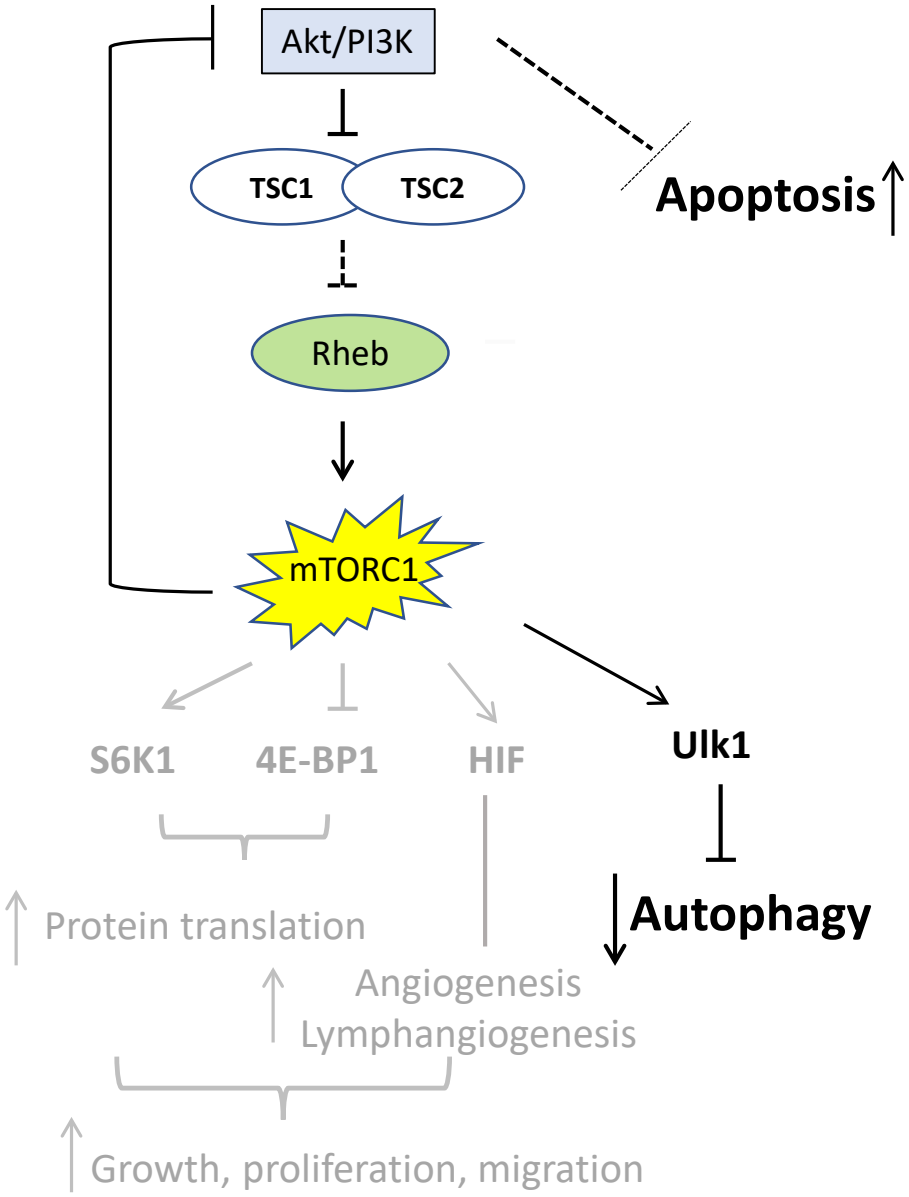
phase 2, open label, single arm

Duration of treatment: 9 months

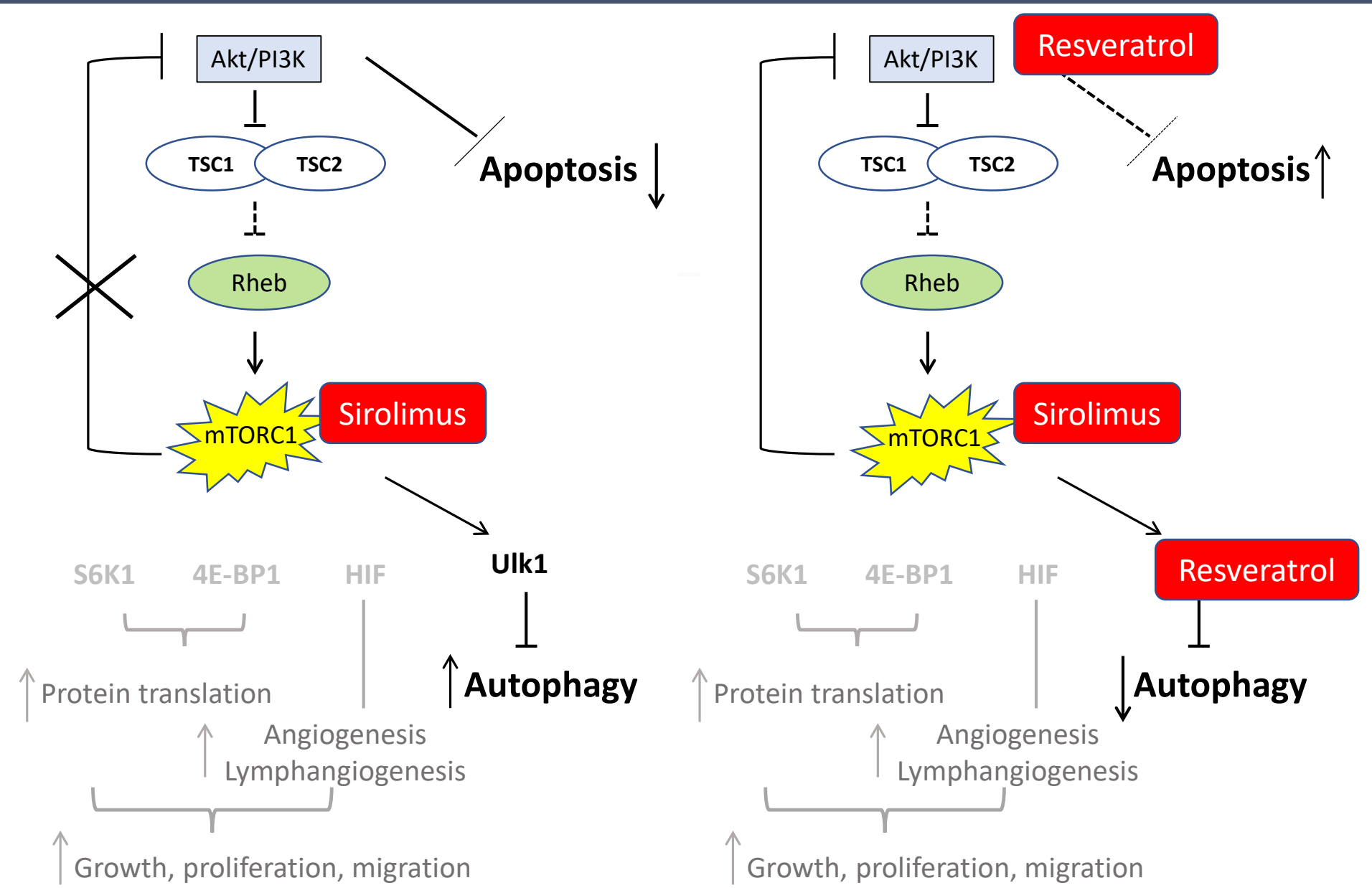
Inclusion criteria: a recent reduction in forced expiratory volume at 1-second (FEV1) of > 50ml/year

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

LAM - Pathogenesis



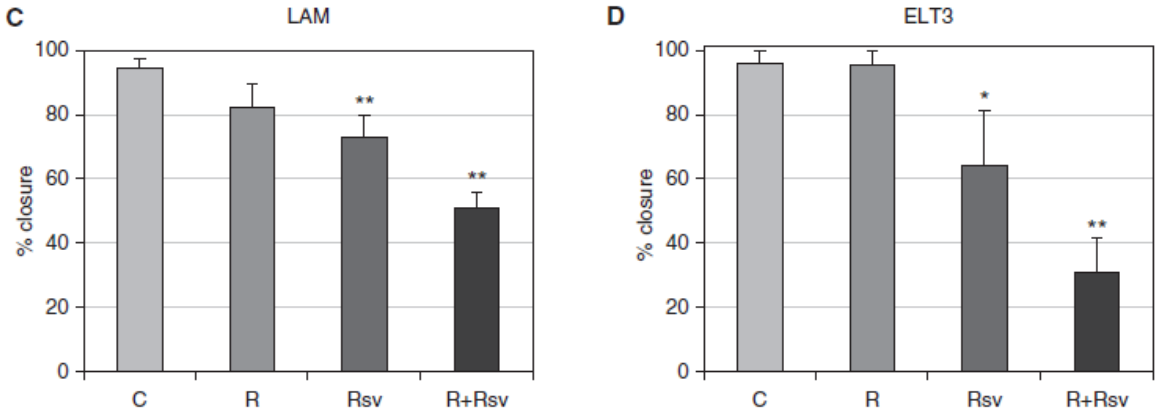
LAM - Pathogenesis

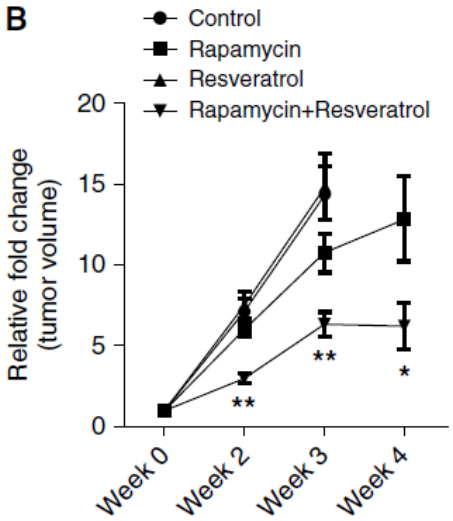


Alayev A et al, Cell cycle 2014

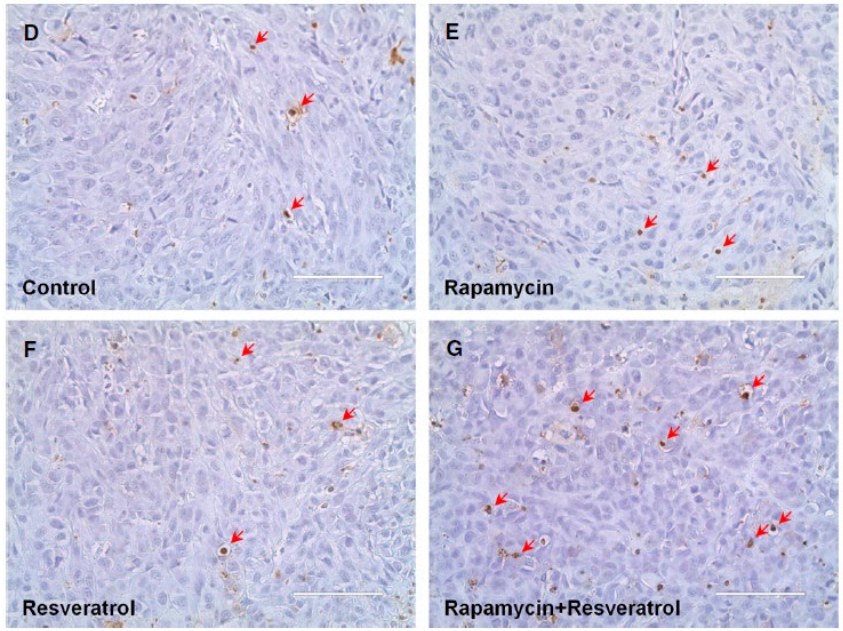
Combination of rapamycin and rasveratrol

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

Ongoing

"RESULT"

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

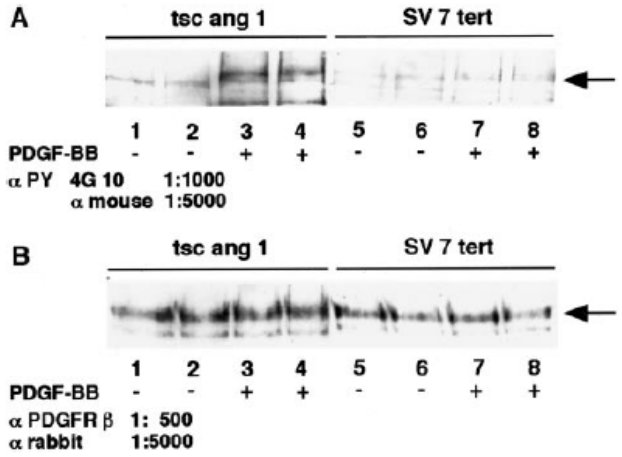
phase 2, open label, single arm,

Duration of treatment: 24 weeks

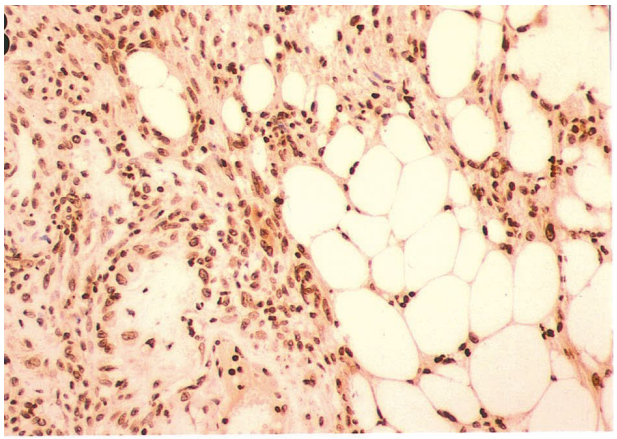
Inclusion criteria: pts with stable disease with sirolimus for at least 20 weeks

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

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

Ongoing

A pilot study of efficacy and safety of nintedanib in LAM

phase 2, open label, single arm

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

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

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

Nintedanib in LAM

Target enrollment: 30 patients
Enrolled to date: 24 patients

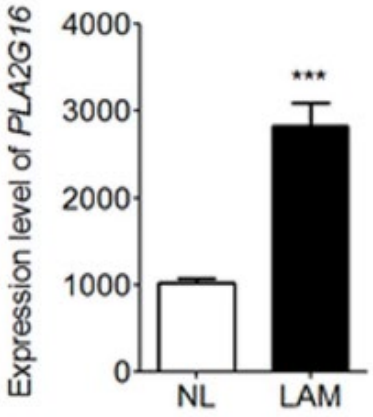
1 patients dropped-out after 4 months because of declining lung function
1 patient dropped-out after 3 months for abdominal pain, diarrhea, increased blood pressure
1 patient dropped-out after 5 months for increased ALT, AST (>5 fold ULN)
1 patient withdrawn consent after 8 months

Side effects in first 11 patients who completed 12 months of treatment:

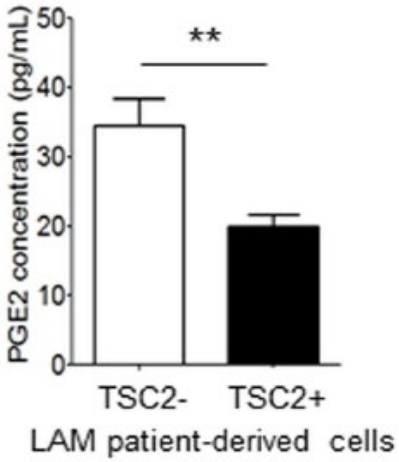
- 1 SAE not related to drug
- 2 pts experienced increased ALT, AST with need of temporary stop (> 3 fold ULN).
- 2 pts experienced increased ALT, AST not clinically significant
- 1 haemorrhagic cystitis
- 1 slight increased of blood pressure
- 1 hypercholesterolemia
- 4 temporary diarrhea
- 1 headache
- 10 patients experienced nausea

Phospholipase A2 (PLA2) in TS and LAM

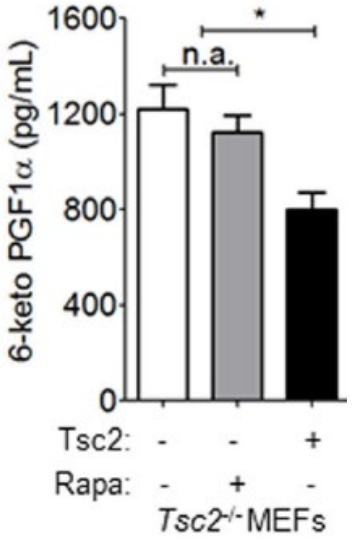
PLA2 is the rate-limiting enzyme that catalyzes the conversion of plasma membrane phospholipids into PGs



AdipocytePLA2is upregulated in lung LAM nodule cells compared to non-LAM

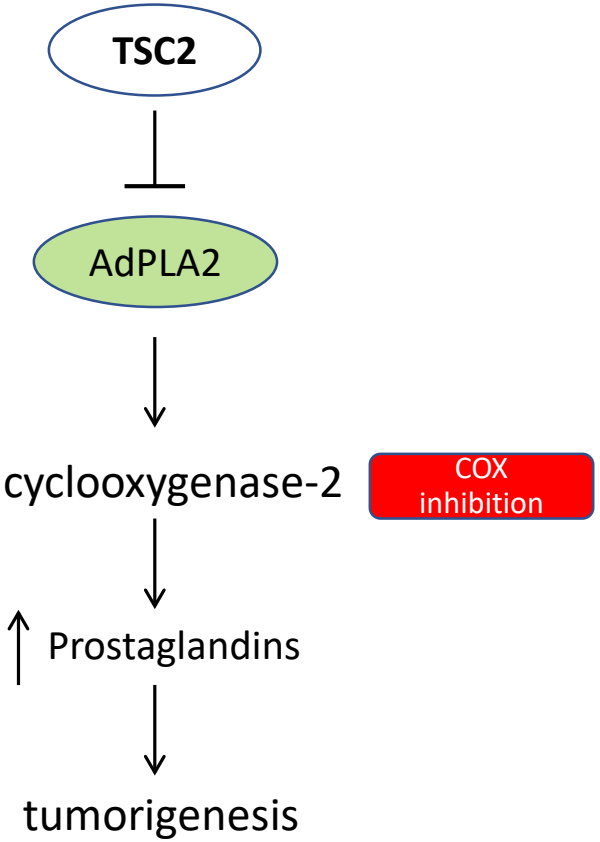


Secreted levels of PGs are higher from TSC2 deficient cells than TSC-addback cells



Secreted levels of PGs metabolite are higher in TSC2 deficient cells and are not affected by rapamycin

Non canonical TSC pathways



Li C et al, PLOS ONE 2014

COX-2 Inhibition in LAM

Ongoing

"COLA"

A Pilot Clinical Trial of COX-2 Inhibition (celecoxib) in LAM and TSC

phase 2, open label, single arm

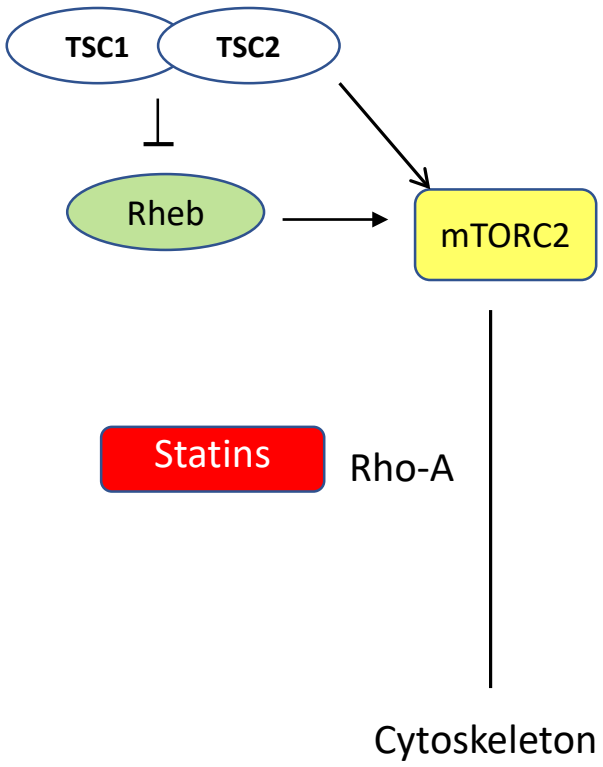
Duration of study: 6 months treatment period, 6 months follow-up

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

mTOR Inhibition and statins in LAM

Non canonical TSC pathways



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

Ongoing

"SOS"

Safety of Simvastatin in LAM and TSC patients on a stable dose of sirolimus or everolimus

phase 1-2, open label, single arm

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

Pathogenesis: role of estrogen

- ✓Female predominance
- ✓Frequent occurrence during childbearing age
- ✓Decline in lung function is greater in pre-menopausal women than in post-menopausal women
- ✓Reported worsening following the administration of estrogens or during pregnancy
- ✓Presence of estrogen receptors in LAM cells

In *in vitro* and animal models estrogen interacts with signaling events

- to stimulate growth of human AML cells
- to promote the survival and pulmonary metastasis of Tsc 2−/− ELT3 cells

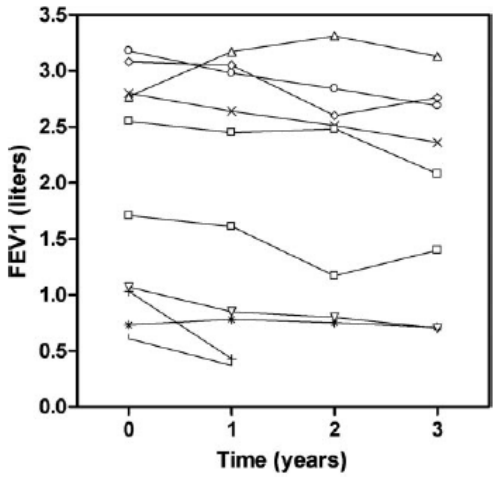
Hormonal treatment

- ✓Oophorectomy, anti-estrogen therapy: controversial effects , no objective evidence of improvement
- ✓Progesterone: case reports, retrospective studies

Taveira–Dasilva AM et al, Chest 2004

- ✓ Gonadotrophin-releasing hormone (GnRH) analogues: case reports, retrospective studies.

A prospective study of triptorelin in 10 patients showed no effects on lung function over a 3-year period



Harari S et al, Chest 2008

Aromatase inhibition in LAM

A phase II clinical trial

Completed

"TRAIL"

Efficacy and safety of the aromatase inhibitor letrozole in postmenopausal women with LAM

Phase2,randomized, double blinded, placebo-controlled

Duration of treatment: 12 months

- Primary endpoint: FEV1
- Secondary endpoints: pulmonary function
  quality of life
  serum VEGFD

The target enrollment of 25 patients per arm was not met

(9 patients received letrozole, 8 patients received placebo)

In a post hoc analysis, eight matched letrozole-treated-placebo-treated pairs were constructed, six of which demonstrated better FEV_1 improvement for the letrozole-treated patients

Lu C et al, Ann Am Thorac Soc. 2017

Clinical trials

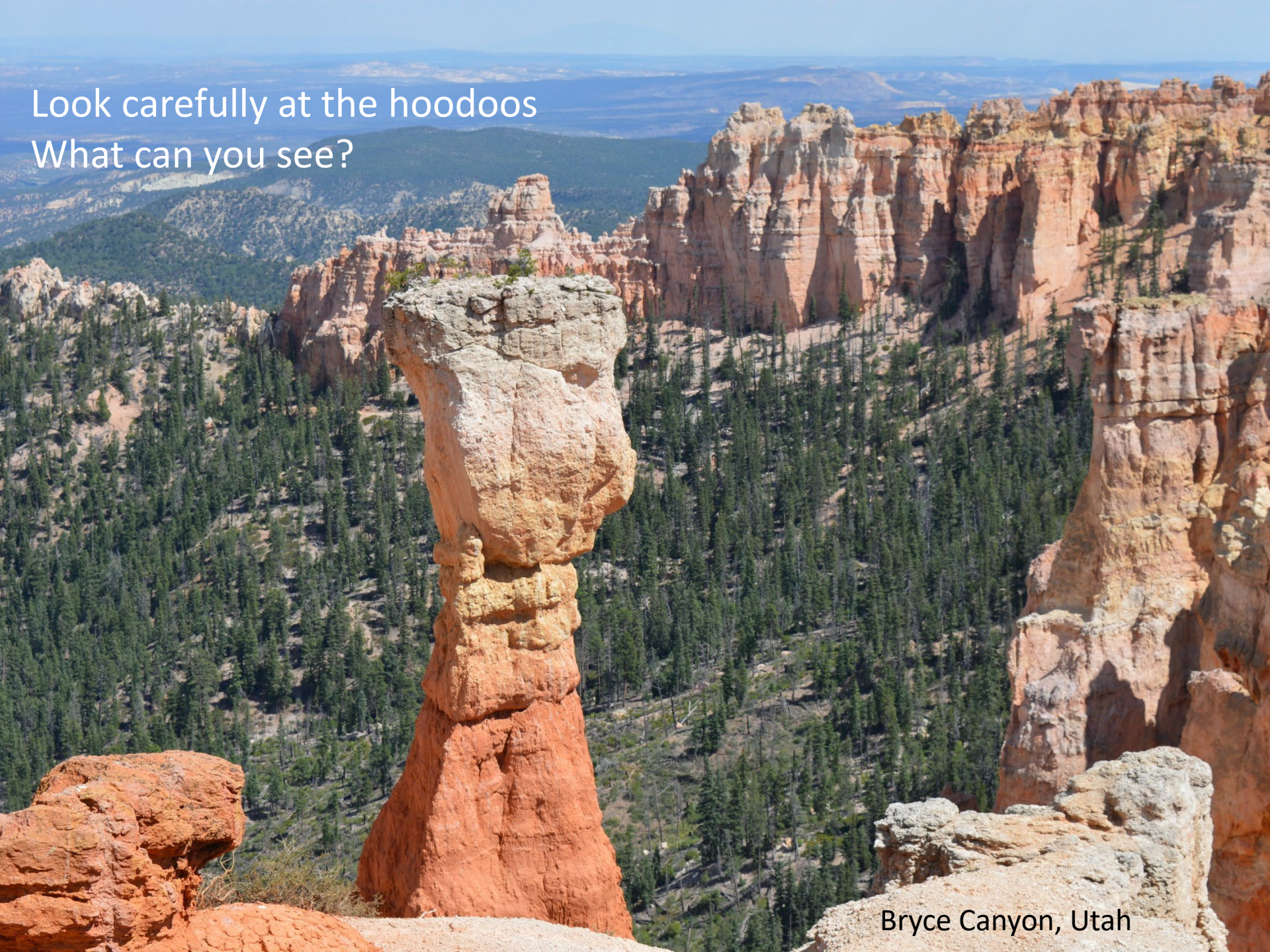
Name	Drug	Design	Primary endpoint	Status	Results
MILES	Sirolimus	Randomized, d. blind, placebo controlled	FEV1	Completed	Positive results
	Everolimus	Open label, dose-escalating	Safety, PK, PD	Completed	Lower safety profile compared to sirolimus
SAIL	Sirolimus plus Hydroxychloroquine	Open label	Safety	Completed	Positive results at higher dose
TRAIL	Letrozole	Randomized, d. blind, placebo controlled	FEV1	Completed	Failed to enrolled the target n of pts
	Doxycycline	Randomized, d. blind, placebo controlled	FEV1	Completed	Negative results
SLAM-2	Saracatinib	Open label, Single arm	FEV1	Ongoing	
LAM	Nintedanib	Open label Single arm	FEV1	Ongoing	
LAMP1	Imatinib	Randomized, blind, placebo controlled	VEGFD	Ongoing	
RESULT	Sirolimus plus Resveratrol	Open label, Single arm	VEGFD	Ongoing	
COLA	Celecoxib	Open label, Single arm	Safety	Ongoing	
SOS	mTOR inhibitors plus simvastatin	Open label Single arm	Safety	Ongoing	

Take home messages

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

2. Alternatives or combined drugs are currently under investigation

3. An individualized combined or multiple steps treatment is likely the future for LAM patients


Look carefully at the hoodoos
What can you see?
Bryce Canyon, Utah