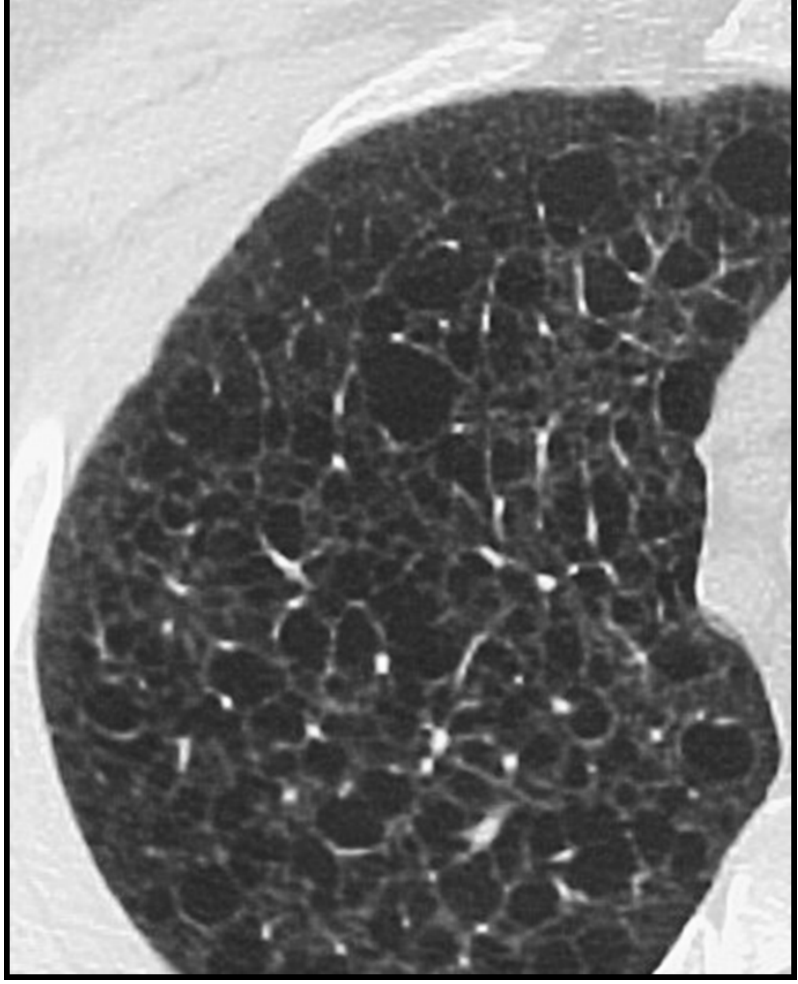


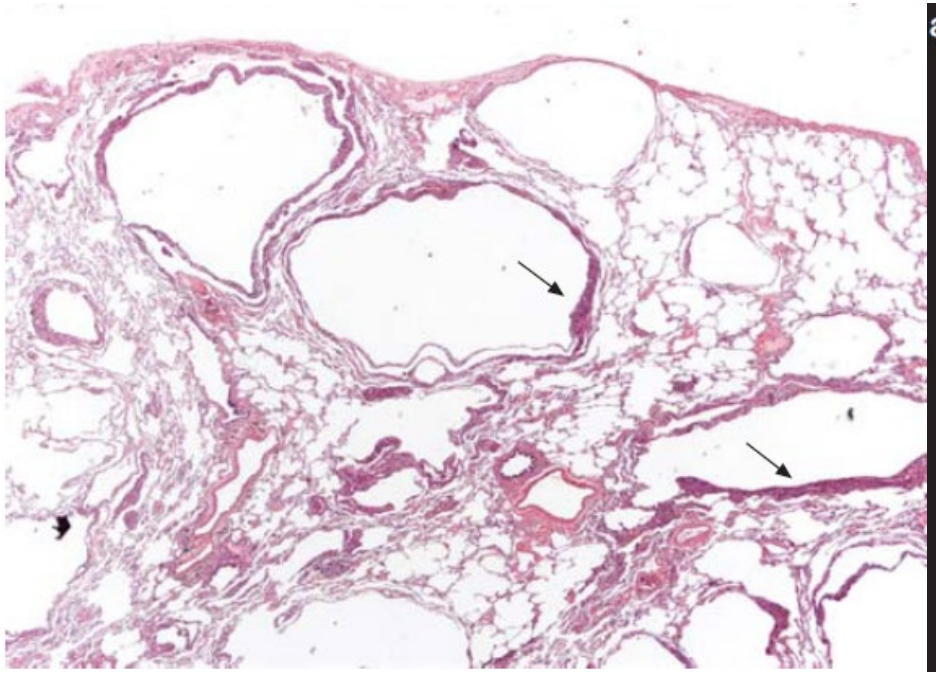
Acquisizioni nella gestione clinica della LAM
Advances in the clinical management of LAM

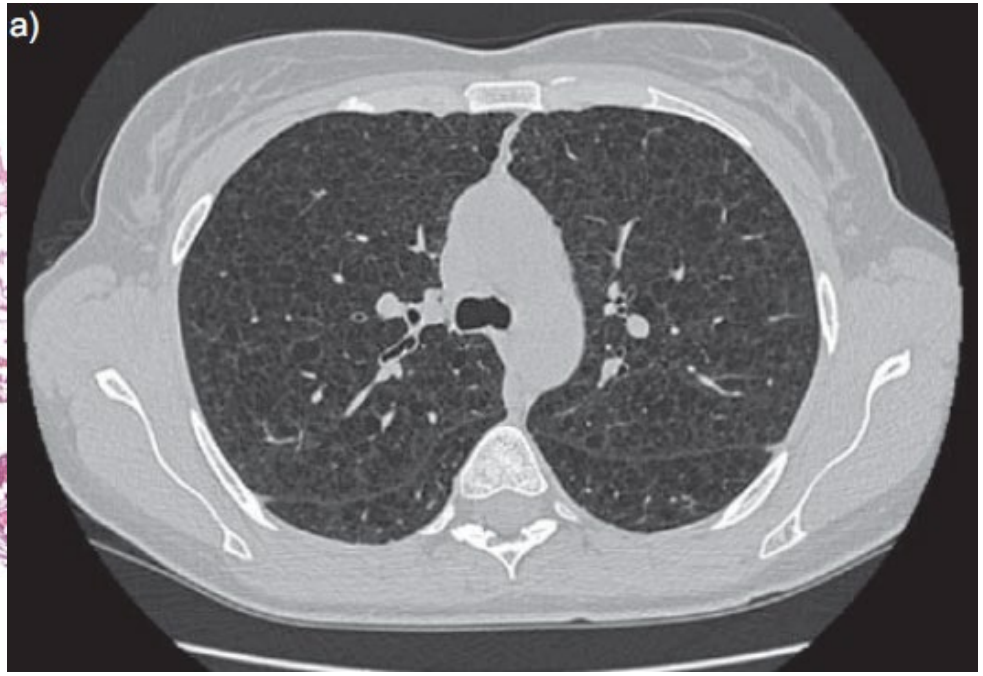
Sergio Harari
U.O. di Pneumologia e UTIR – U.O. di Medicina Interna
Ospedale San Giuseppe – MultiMedica IRCCS - Milano

- What is LAM
- Diagnosis
- Treatment

- What is LAM
- Diagnosis
- Treatment

Lymphangioleiomyomatosis (LAM)





Lymphangioleiomyomatosis (LAM) is a rare multisystem disorder affecting predominantly young females in their reproductive years. It is characterised by progressive cystic destruction of the lung, lymphatic abnormalities and abdominal tumours(e.g. angiomyolipomas)

LAM: a tumor

LAM pathogenic mechanisms mirror those of many forms of human cancer

- ❖ Mutations
- ❖ Inappropriate growth and survival
- ❖ Metastasis via blood and lymphatic circulation
- ❖ Infiltration
- ❖ Tissue destruction
- ❖ Sex steroid sensitivity

LAM has been included in PEComas

But the source of LAM cells is still unknown

(Uterus? Kidneys? Lymphatics?)

LAM cells show no atypia

Henske EP, McCormack FX AJRCCM 2012
Harari S et al, ERJ 2015

- What is LAM
- **Diagnosis**
- Treatment

Diagnosis – ERS guidelines

Definite LAM	characteristic lung HRCT + any of the following - angiomyolipoma - thoracic or abdominal chylous effusion, - lymphangioleiomyoma - biopsy-proven lymph node involved by LAM - TSC
Probable LAM	characteristic lung HRTC + compatible clinical history compatible lung HRTC + angiomyolipoma or chylous effusion
Possible LAM	characteristic or compatible lung HRTC alone

Characteristic HRCT: multiple (more than 10) thin-walled round well-defined air-filled cysts with no other significant pulmonary involvement (with the exception of MMPH in TSC)

Compatible HRTC: few (more than two and fewer than 10) typical cysts

Johnson SR et al, ERJ 2010

LAM – Biomarkers
VEGF-D

2006	Seyama K et al.	VEGF-D is increased in serum of patients with LAM
2008	Young et al.	VEGF-D serum levels are higher in LAM than in similar cystic or chylous lung diseases
2009	Glasgow et al	VEGF-D levels in LAM reflect lymphatic involvement
2010	**Young et al.**	**VEGF-D level higher than 800 pg/mL in a woman with typical changes on high-resolution CT scan is diagnostically specific for LAM, and identifies LAM in women with TSC**
2013	Young et al.	The results of an analysis of data from the MILES trial confirm that VEGF-D is a useful biomarker that correlates with disease severity and treatment response

2016

Official American Thoracic Society/Japanese Respiratory Society Clinical Practice Guidelines: Lymphangioleiomyomatosis Diagnosis and Management

Francis X. McCormack, Nishant Gupta, Geraldine R. Finlay, Lisa R. Young, Angelo M. Taveira-DaSilva, Connie G. Glasgow, Wendy K. Steagall, Simon R. Johnson, Steven A. Sahn, Jay H. Ryu, Charlie Strange, Kuniaki Seyama, Eugene J. Sullivan, Robert M. Kotloff, Gregory P. Downey, Jeffrey T. Chapman, MeiLan K. Han, Jeanine M. D'Armiento, Yoshikazu Inoue, Elizabeth P. Henske, John J. Bissler, Thomas V. Colby, Brent W. Kinder, Kathryn A. Wikenheiser-Brokamp, Kevin K. Brown, Jean F. Cordier, Cristopher Meyer, Vincent Cottin, Jan L. Brozek, Karen Smith, Kevin C. Wilson, and Joel Moss; on behalf of the ATS/JRS Committee on Lymphangioleiomyomatosis

VEGF-D testing is recommended to establish the diagnosis of LAM

LAM diagnosis

Definite LAM: characteristic lung HRCT + any of the followings

ERS guidelines 2010	ATS/JRS guidelines 2016
- Tuberous Sclerosis Complex	✓
- Chylous effusions	✓
- Angiomyolipomas	✓
- Lymphatic involvement	✓
	- Serum VEGFD levels ≥ 800 pg/mL
	- Histopathological confirmation is indicated when characteristic HRTC is present with no additional confirmatory characteristics

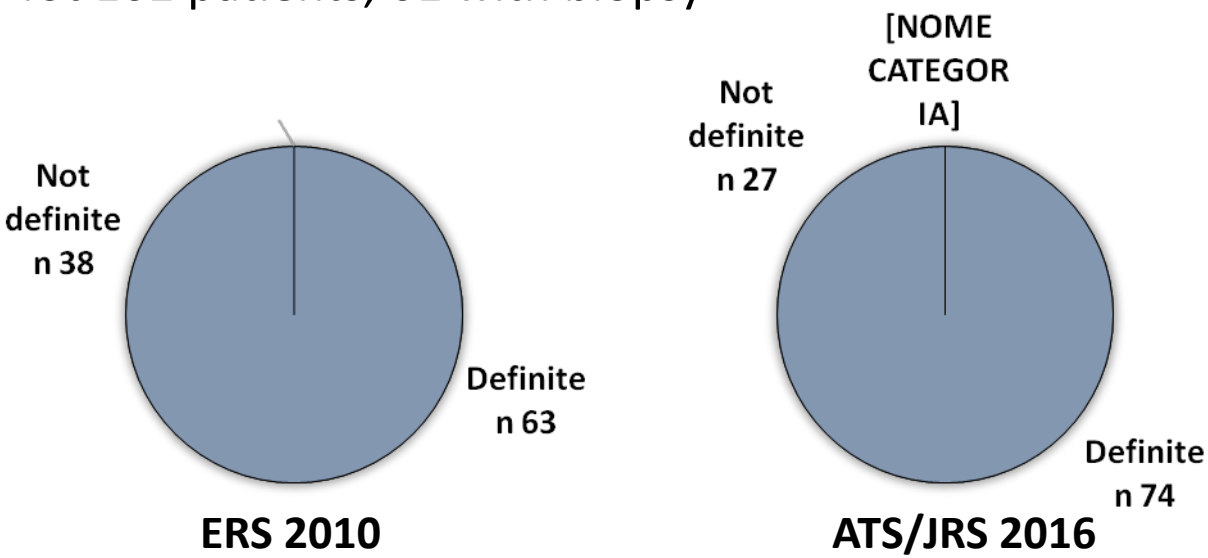
Johnson SR et al, ERJ 2010
McCormack FX et al, AJRCCM 2016

VEGF-D

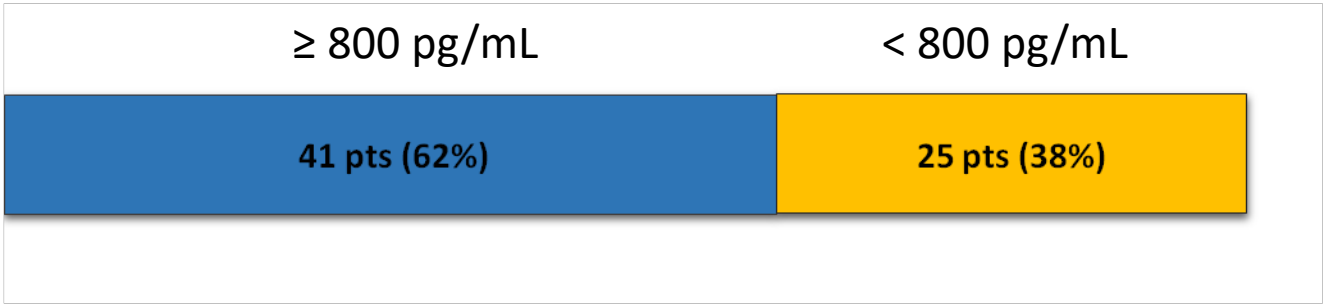
Ospedale San Giuseppe

Tot 162 patients, 61 with biopsy

101 patients without biopsy



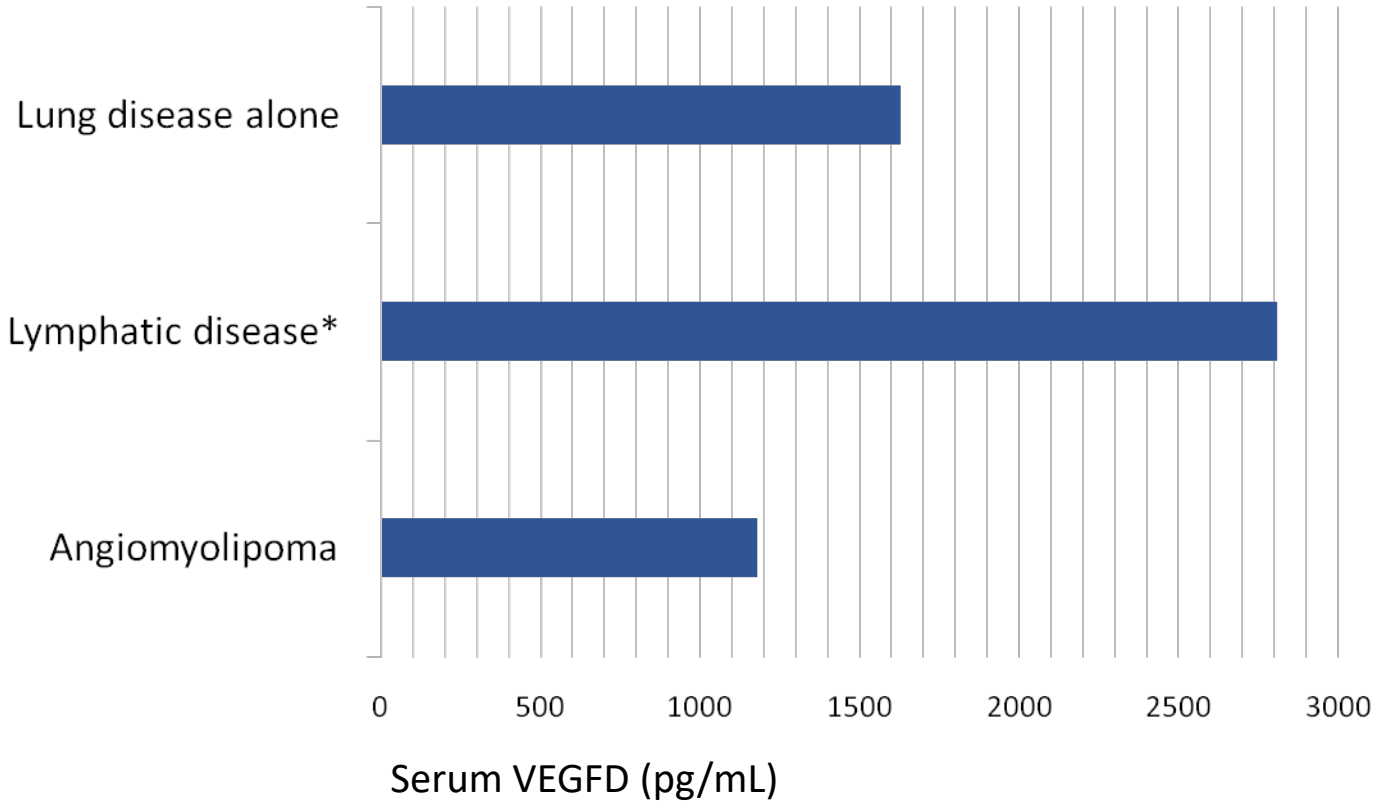
VEGFD is avalaible in 66 patients with definite LAM according to ERS Guidelines or biopsy



With a threshold of 800pg/mL, the reported sensitivity of VEGFD is 70%

VEGF-D

Ospedale San Giuseppe

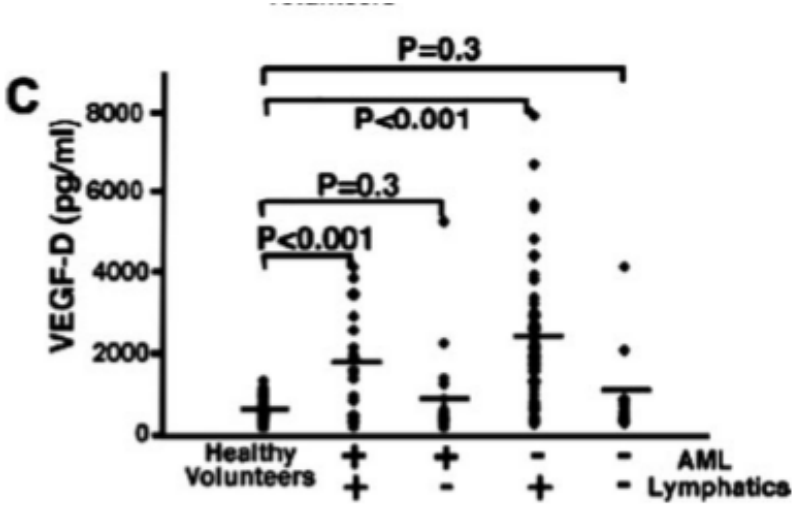


* Chilous effusions, lymph nodes, lymphangioleiomyomas

LAM – Biomarkers
VEGF-D

- Serum VEGFD testing has a low false positive rate but a high false negative rate: a serum VEGFD value < 800 pg/mL does not exclude LAM.

- Serum VEGFD can vary according to the disease manifestations.
  It is usually higher in patients with lymphatic manifestations.



Glasgow CG et al, Chest 2009

New biomarkers are still needed for diagnosis, follow-up, and designing of clinical trials

Other biomarkers?

- Serum and/or urinary levels of MMPs

  - LAM nodules have been shown to contain MMP activators and inhibitors

  - MMPs have been implicated in the pathogenesis of LAM

  - Serum and urinary levels of MMP-9 have been found to be higher in patients with LAM than in normal subjects

  - MMPs are not specific

Hayashi T et al, Human Pathol 1997
Odajima N et al, Respir Med 2009

- TSC loss of heterozygosis (LOH) in cells from body fluids

  - TSC LOH is involved in LAM pathogenesis

  - LAM cells, identified by TSC2 LOH, have been isolated from the blood and other body fluids of LAM patients and detection is reduced after treatment with sirolimus.

  - The search for circulating LAM cells in blood or other fluids may identify patients at risk of disease progression or spread and/or the response to potential therapy

Crooks et al, PNAS 2004
Cai et al, Chest 2014

SAMPLE	BLOOD	URINE
O.Torre 5	▲	
O.Torre 11	▲	
O.Torre 12	▲	
O.Torre 15	▲	
O.Torre 16	□	
O.Torre 18	▲	
O.Torre 19	▲	
O.Torre 20	▲	
O.Torre 21	▲	
O.Torre 22	▲	
O.Torre 23	▲	
O.Torre 24	▲	□
O.Torre 25	□	
O.Torre 27	▲	
O.Torre 28	▲	
O.Torre 29	▲	▲
O.Torre 30	▲	
O.Torre 34	▲	
O.Torre 35	▲	□
O.Torre 37	▲	▲
O.Torre 39	▲	
O.Torre 40	▲	
O.Torre 41	▲	▲
O.Torre 42	▲	□
O.Torre 43	▲	□
O.Torre 45	▲	▲
O.Torre 47	□	
O.Torre 49	▲	
O.Torre 51	▲	□
O.Torre 52	▲	□
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O.Torre 57	▲	
O.Torre 58	▲	
O.Torre 59	▲	▲
O.Torre 61	▲	
O.Torre 62	▲	□
O.Torre 63	▲	
O.Torre 64	▲	
O.Torre 68	▲	▲
O.Torre 70	▲	▲
O.Torre 71	□	□
O.Torre 75	▲	□
O.Torre 78	▲	
O.Torre 80	▲	
O.Torre 82	▲	
O.Torre 85	□	□
O.Torre 86	▲	□
O.Torre 87	▲	
O.Torre 88	▲	□
O.Torre 89	▲	
O.Torre 92	▲	

TSC LOH

Ospedale San Giuseppe

▲ LOH

□ ROH

Tot 53 patients

Blood (CD45-/CD235-, CD45-/CD235+): 91% (48/53)

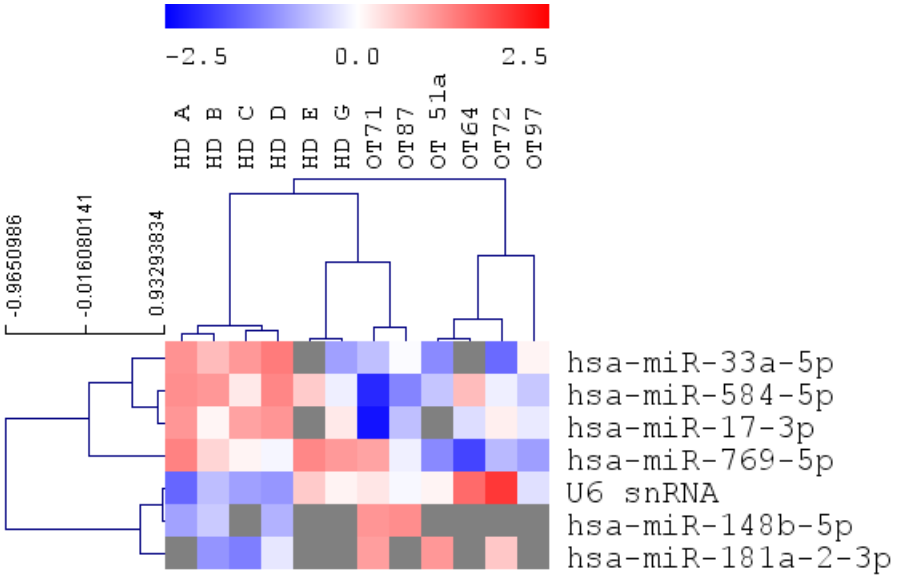
Urine (CD44v6+/CD9+): 32% (7/22)

Ongoing

Search for TSC LOH in different cystic lung diseases to evaluate its possible role as a diagnostic biomarker

miRNAs

Serum microRNAs (miRNAs) have been documented as novel non-invasive biomarkers in a variety of pathological conditions



5 miRNAs resulted to be differentially expressed in the serum samples from LAM patients (OT) compared with controls (HD)

We conducted a preliminary study to evaluate serum miRNoma involving 7 histologically proven LAM patients and 7 matched healthy controls

The validation phase is ongoing

unpublished data

- What is LAM
- Diagnosis
- Treatment

Hormonal therapy

- ✓Estrogen receptors are present in LAM cells
- ✓Reports of disease progression during pregnancy or following exogenous estrogen administration
- ✓These findings suggest the involvement of estrogens in the pathogenesis of LAM.

Hormonal therapy

- ✓ Oophorectomy
- ✓ Anti-oestrogen therapy

Controversial effects
No objective evidence of improvement

- ✓ Gonadotrophin-releasing hormone (GnRH) analogues

Case reports
Retrospective studies
A prospective study showing no effects on lung function

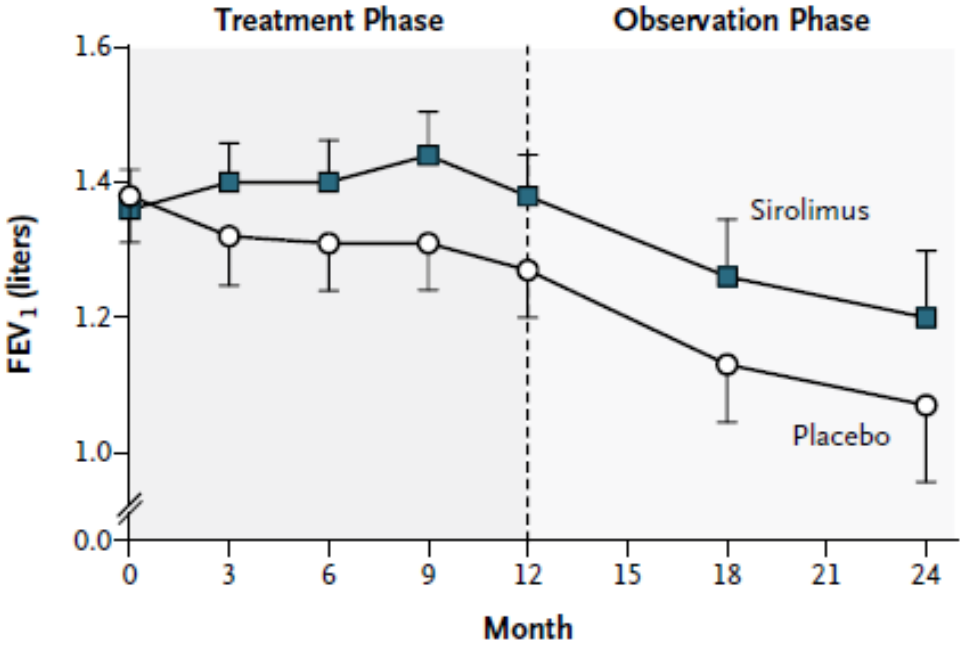
- ✓ Progesterone

Case reports
Retrospective studies with controversial results

Taveira–Dasilva AM, Chest 2004
Harari S, Chest 2008
Lu C, Ann Am Thorac Soc. 2017

The MILES trial: a milestone

- Stabilization of lung function during the treatment period
- After discontinuation of sirolimus, the decline in lung function resumed and paralleled that in the placebo group



More common adverse effects:
Mouth ulcers, diarrhea, upper respiratory infections, hypercholesterolemia, acneiform rash

McCormack FX et al, NEJM 2011

mTor inhibitors: sirolimus

- Reduction of AMLs volume during the treatment period

  Bissler JJ et al, NEJM 2008

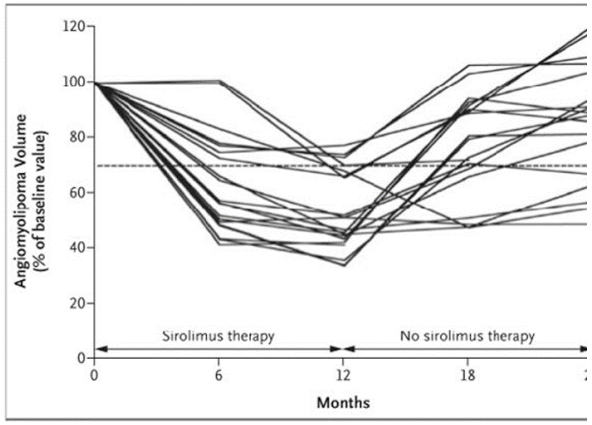
- Stabilization of lung function (FEV1) during the treatment period (MILES trial)

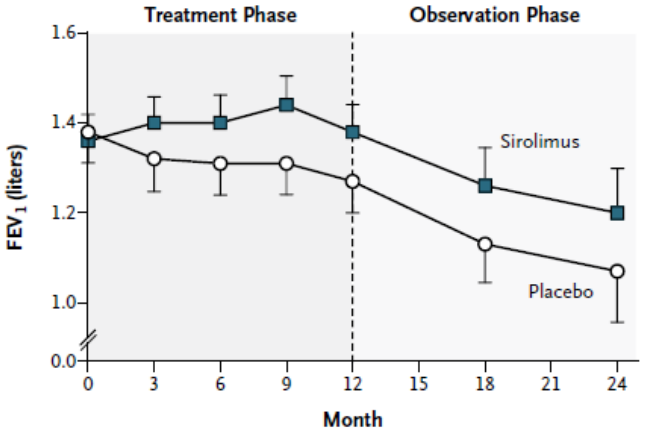
  McCormack FX et al, NEJM 2011

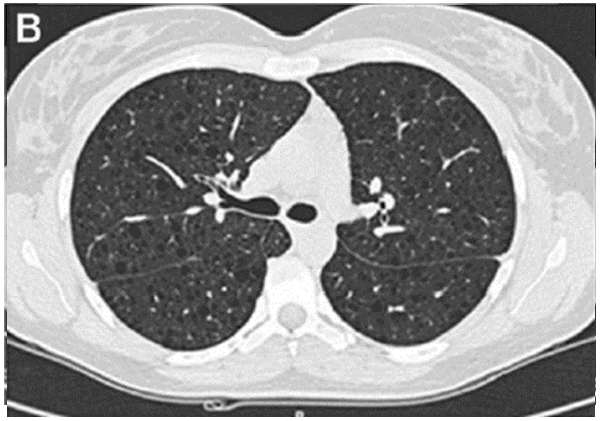
- Resolution of chylous effusions

  Reduction of lymphangioleiomiomas

  Taveira-Dasilva AM, Ann Intern Med. 2011
  Taveira-Dasilva AM, Chest 2018

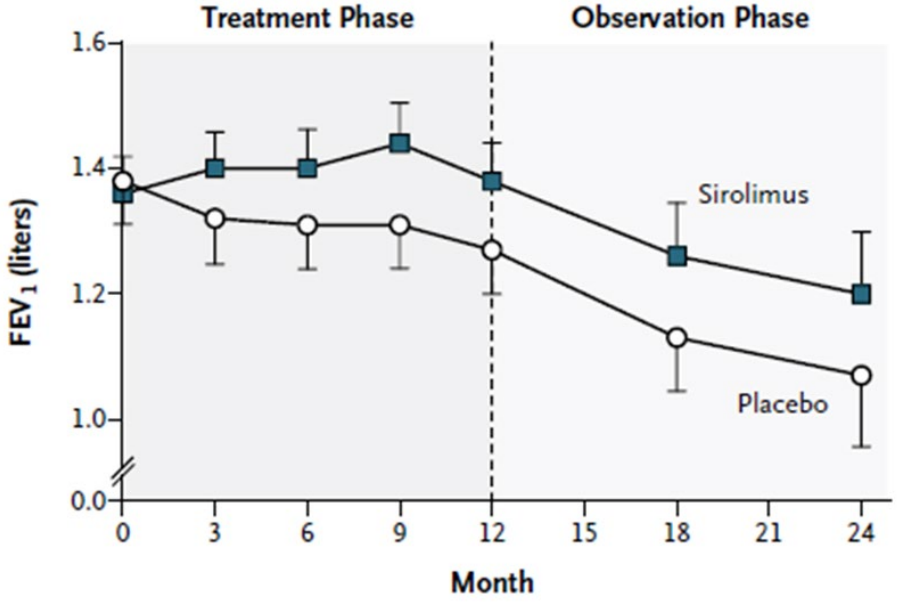






mTor inhibitors: sirolimus

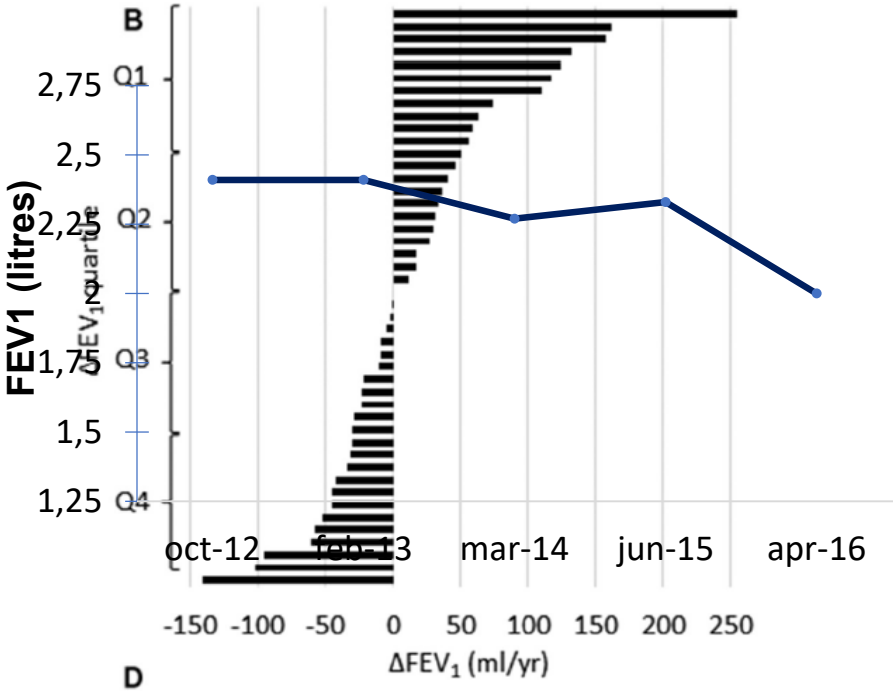
MILES trial,
12 months



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

McCormack FX et al, NEJM 2011

A prospective national cohort study,
35,8 months



Decline of FEV1 after an initial stabilization during treatment with sirolimus

mean treatment ΔFEV1 was 1 ml/y in some patients, ΔFEV1 continues to fall at an accelerated rate.

Personal observation, 2018.

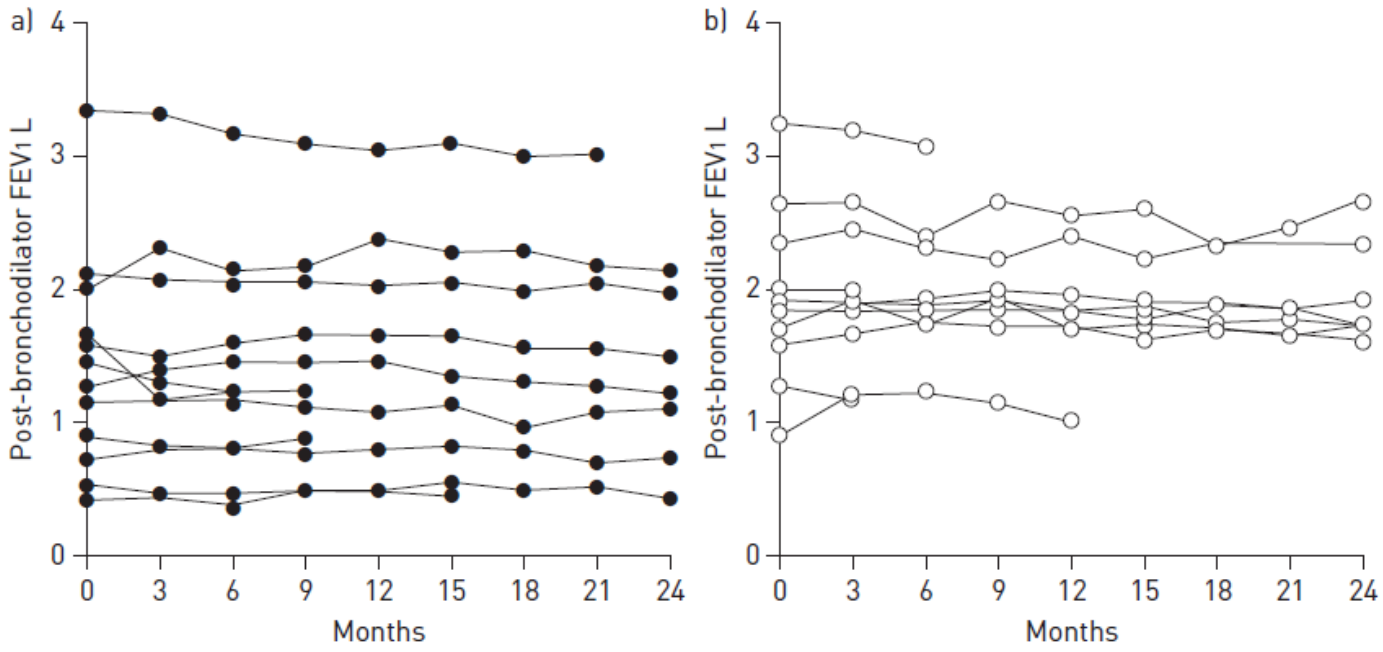
Doxycycline

Metalloproteinases (MMPs) are present within LAM lesions and may have a role in the pathogenesis of cystic lung destruction

Doxycycline, an inhibitor of MMPs, has been hyphotesized as a possible treatment for LAM

Doxycycline

A 2-year randomised placebo-controlled trial



Primary endpoint: no difference in rate of decline in postbronchodilator FEV1

Secondary endpoints: no difference in FVC, DLCO, WD, quality of life scores, VEGF-D

Chang W, ERJ 2014

LAM treatment

ERS guidelines 2010

- Sirolimus only in clinical trials or on an individual basis
- Intramuscular progesterone may be trialled in patients with a rapid decline in lung function or symptoms
- Other hormonal treatments are not recommended

ATS/JRS guidelines 2016

- Sirolimus is recommended
- Hormonal therapy is not recommended
- Doxycycline is not recommended

Johnson SR et al, ERJ 2010
McCormack FX et al, AJRCCM 2016

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: current indications

ATS/JRS Guidelines

- Patients with abnormal lung function

Abnormal lung function: FEV1 less than 70% predicted

Could elevated RV, reduced DLCO, and exercise induced desaturation been considered as criteria to start treatment?

- Patients whose funtion is declining

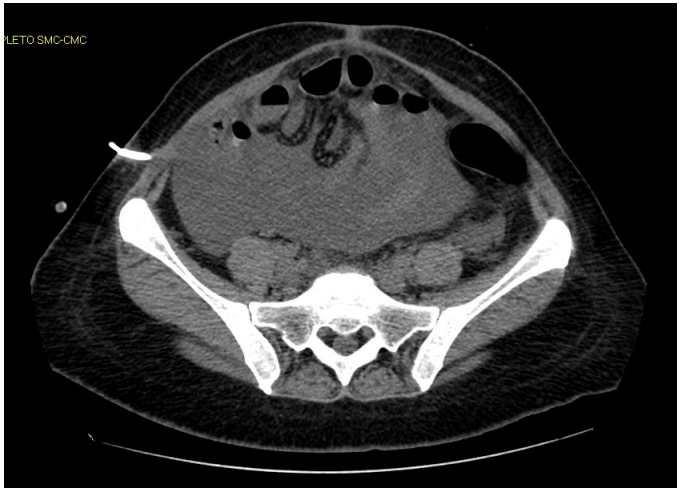
Declining lung function? 90 mL/year?

- Problematic chylous effusions and lymphangioleiomiomas
- Angiomyolipomas?

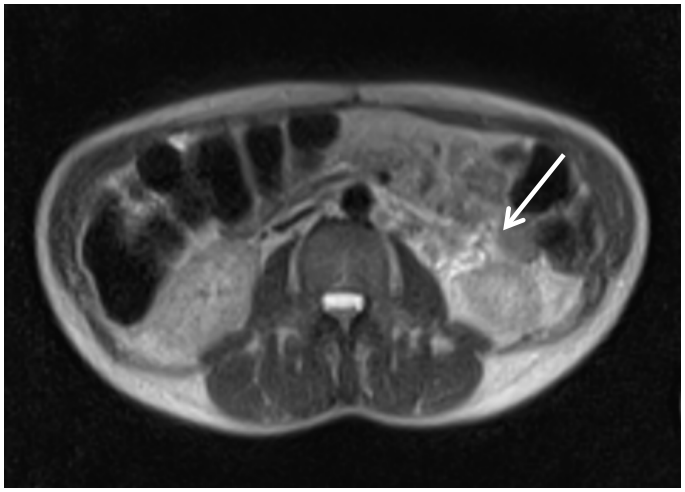
Harari S et al, AJRCCM 2017

Effectiveness of sirolimus in LAM

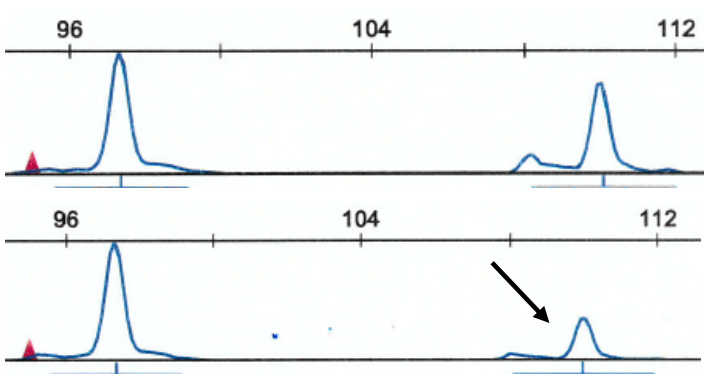
Before sirolimus

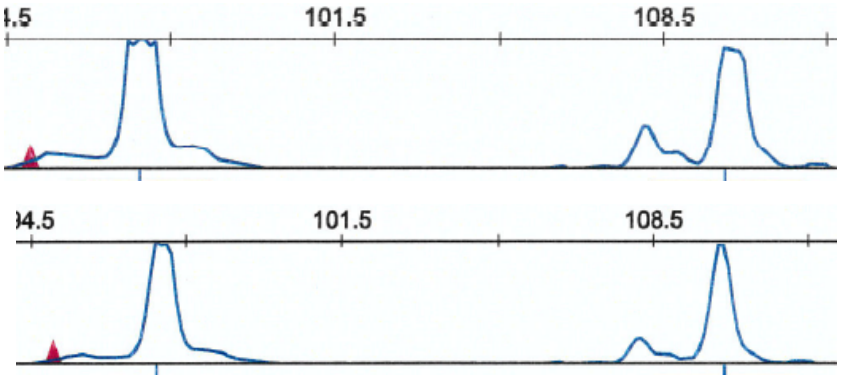


After 3 months of sirolimus



Serum VEGF-D: 4490 pg/mL ⟶ 1 558 pg/mL





Harari S et al, Chest 2016

Everolimus in LAM - Study Design

An *open-label, non randomized, within-patient multiple dose escalation trial*

24 patients enrolled

Primary endpoints: safety and tolerability
Secondary endpoints: lung function

Side effects were generally consistent with known toxicities of mTOR inhibitors
4 serious adverse events

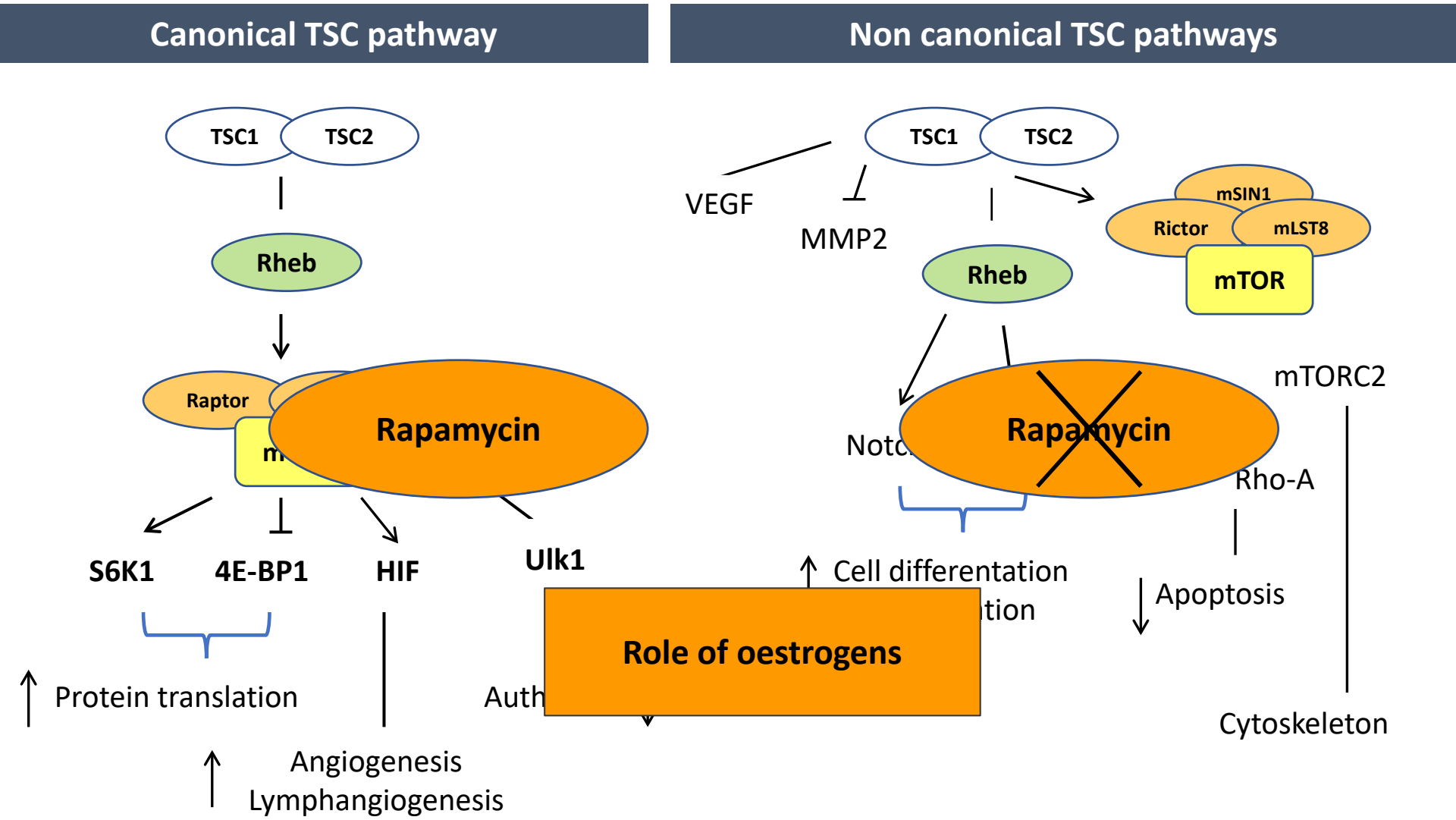
- peripheral oedema
- Pneumonia
- cardiac failure
- Pneumocystis jirovecii infection

were suspected to be related to study drug

After 26 weeks: stability of FVC and improvement of FEV1 compared with baseline

Goldberg HJ, Harari S et al, ERJ 2015

LAM pathogenesis



Treatment: the future

- Combination of mTOR and Autophagy inhibition

- Kinase inhibitors
  - Nintedanib (inhibitor od VEGF-R, PDGF-R, FGF-R)
    Involved mechanism: tumor angiogenesis
  - Saracatinib (inhibitor of Src)
    Involved mechanism: proliferation, motility

- Combination of mTOR inhibition and statins Involved mechanism: apoptosis

- A retrospective study did not show that simvastatin enhances the effects of sirolimus
- A trial of mTOR inhibitors and simvastin is ongoing

Taveira da Silva et al, Chest 2015

- Estrogen antagonism

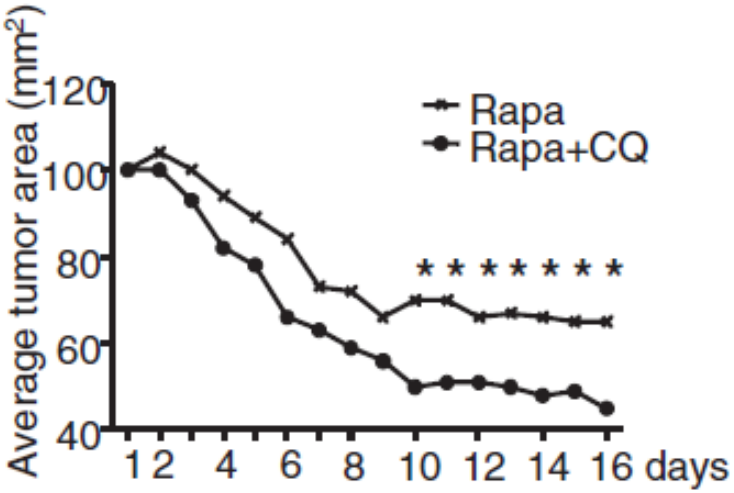
- A randomized, placebo-controlled trial failed to assess effect of letrozole because the target number of enrolled patients was not met

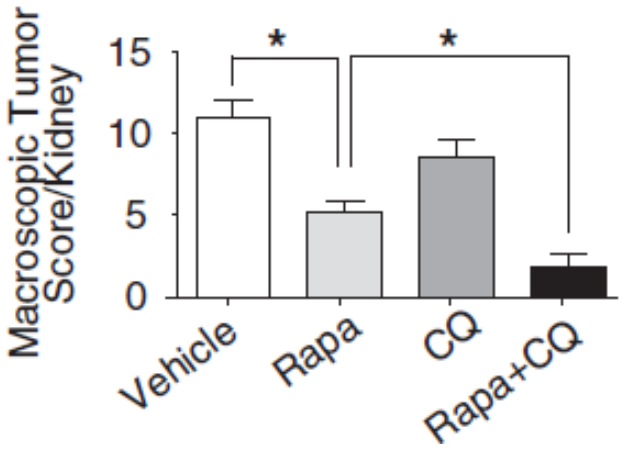
Lu C et al, Ann Am Thorac Soc 2017

- Inhibition of MMPs and other proteases
- Anti-VEGF therapies

Inhibition of autophagy and mTORC

Autophagy has been shown to be a critical component of TSC tumorigenesis





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

Inhibition of autophagy and mTORC

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

24 weeks treatment phase followed by 24 weeks observation phase
13 patients enrolled
8 patients completed both study phases

Primary end point: - Most common adverse events: mucositis, headache, diarrhea
- No drug-related SAEs

Secondary end points:
Entire cohort

End of treatment End of observation

Variable	Baseline[a]	Value at 24 Wk[a]	*P* Value[b]	Value at 48 Wk[a]	*P* Value[c]
FEV_1,[d] mL	1,772 ± 183	1,892 ± 184	.0323	1,755 ± 186	.0303
FVC,[d] mL	2,933 ± 174	3,091 ± 178	.13	3,047 ± 185	.7122
D_{LCO} (mL/mm Hg/min)	9.79 ± 0.93	10.2 ± .94	.0989	9.83 ± .95	.1766
6MWD	442.5 ± 27.0	484.7 ± 27.3	.0003	460.9 ± 27.7	.0391
SGRQ	43.81 ± 5.41	41.92 ± 5.44	.4183	43.36 ± 5.58	.5899
LogVEGF-D	3.12 ± 0.09	2.91 ± 0.09	< .0001	3.08 ± 0.09	< .0001

Cohort of patients receiving 400mg hydroxychloroquine: FEV1 and FVC remained stable at 48 weeks

El-Chemaly S et al, Chest 2017

A pilot study of nintedanib for lymphangioleiomyomatosis

A non-randomized, efficacy, safety, and tolerability trial of nintedanib in sporadic and TSC-associated LAM

Objective To demonstrate the efficacy and safety of nintedanib in the treatment of LAM patients with progressive disease

Study design Single Group Assignment
Open Label

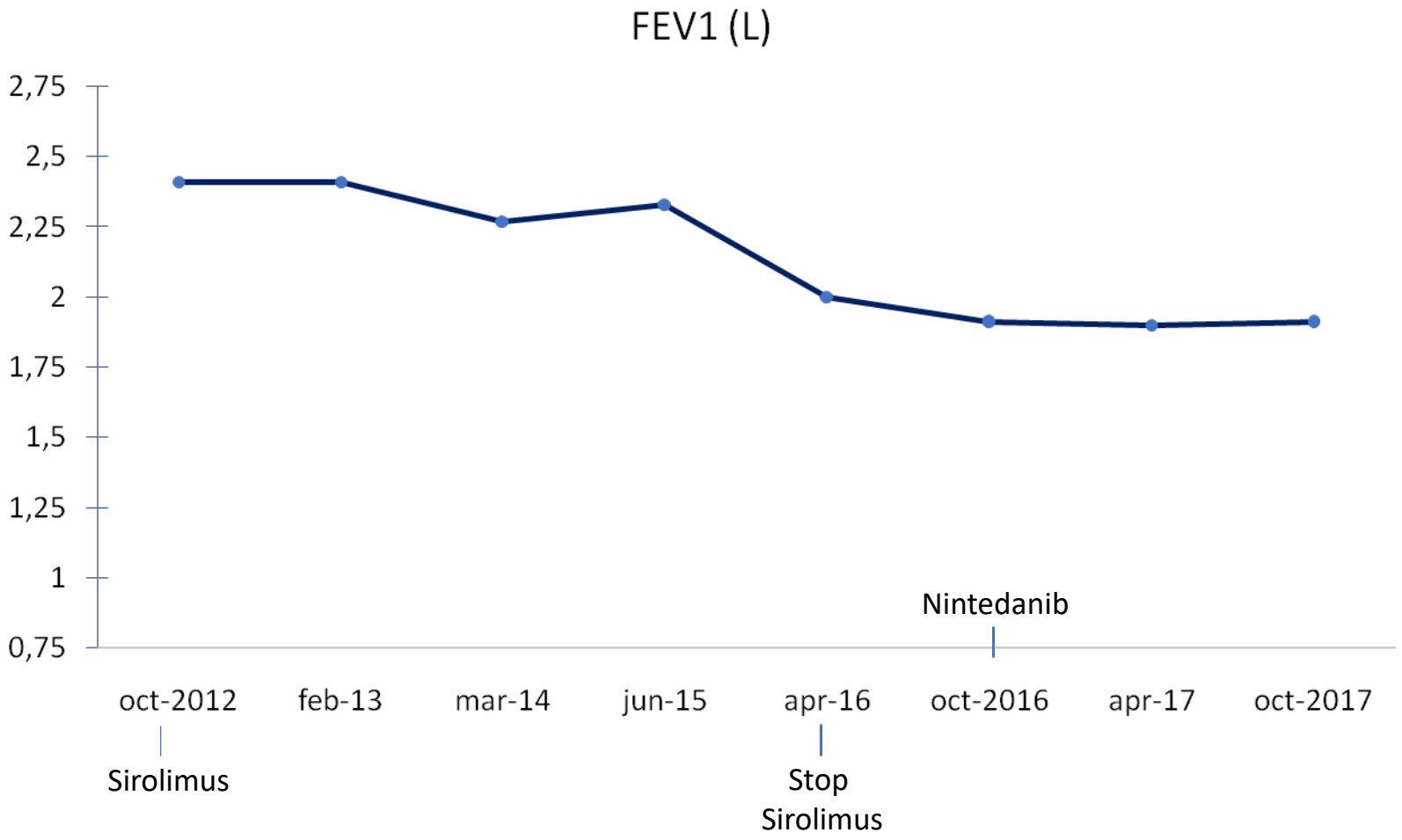
30 patients (28 patients enrolled)
12 months treatment period
12 months follow-up

Primary endpoint: FEV_1 response, assessed as the change in FEV_1 (FEV_1 slope) in milliliters per month

Rationale inhibition of PDGF, whose receptor is present and active in human TSC lesions
inhibition of tumor angiogenesis which is regulated by VEGF, PDGF, and FGF signaling pathways

A 31 years old woman with a biopsy proven LAM started sirolimus in october 2012

After an initial stabilization with therapy, FEV1 started declining



A pilot study of nintedanib for lymphangioleiomyomatosis

SAFETY DATA IN 10 PATIENTS THAT COMPLETED 12 MONTHS OF THERAPY

- No SAE
- 2 pts experienced increased ALT, AST with need of temporary stop (> 3 fold ULN).

After decrease of values, they restarted therapy at 100mg bid.

- 2 pts experienced increased ALT, AST not clinically significant
- 1 haemorrhagic cystitis
- 1 slight increased of blood pressure
- 1 slight hypercholesterolemia
- 3 temporary diarrea
- 1 headache
- 9 patients experienced nausea

Treatment: the future

- Combination of mTOR and Autophagy inhibition
- Kinase inhibitors
- Combination of mTOR inhibition and statins
- Estrogen antagonism
- Biguanides

 - Biguanides function as activators of adenosine monophosphate-activated protein kinase (AMPK), which in turn strongly suppresses mTOR

 - The use of an AMPK activator alone or in combination with an mTOR inhibitor may be a promising choice for the treatment of various cancers

 - Metformin has recently been studied in a randomized controlled trial in tuberous sclerosis complex-related angiomyolipomas,

Conclusions

1.

LAM is a neoplastic disease

2.

Biomarkers for diagnosis and follow-up still represent a main issue. Work in progress

3.

An individualized treatment is likely the future for LAM patients