PNEUMOLOGIA 2016

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Le patologie cistiche polmonari: cosa c'è di nuovo?

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Diffuse cystic lung disease (DCLD)

- DCLDs are characterized by the presence of multiple spherical or irregularly shaped, thinwalled, air filled spaces within the pulmonary parenchyma.

- In most cases lung remodeling associated with inflammatory or infiltrative processes results in displacement, destruction or replacement of alveolar septa, distal airways and small vessels within the secondary lobules of the lung.

Classification of DCLDs

1. *Neoplastic*	**Lymphangioleiomyomatosis** (S-LAM or TSC-LAM) **Pulmonary Langerhans cell histiocytosis**, and non-Langerhans cell histiocytoses including Erdheim Chester disease Other primary and metastatic neoplasms such as sarcomas, adenocarcinomas, pleuropulmonary blastoma, etc.
2. Genetic Developmental Congenital	Birt-Hogg-Dubé syndrome Proteus syndrome, neurofibromatosis, Ehlers-Danlos syndrome Congenital pulmonary airway malformation, bronchopulmonary dysplasia,etc.
3. *Associated with lympho-proliferative disorders*	Lymphocytic interstitial pneumonia Follicular bronchiolitis Sjögren syndrome Amyloidosis Light chain deposition disease
4. *Infectious*	Pneumocystis jiroveci Staphylococcal pneumonia Recurrent respiratory papillomatosis Endemic fungal diseases Paragonimiasis
5. *Associated with interstitial lung diseases*	Hypersensitivity pneumonitis Desquamative interstitial pneumonia
6. *Smoking related*	Pulmonary Langerhans cell histiocytosis Desquamative interstitial pneumonia
7. *Other/ Miscellaneous*	Post-traumatic pseudocysts Fire-eater's lung Hyper IgE syndrome
8. *DCLD mimics*	Emphysema Alpha-one antitrypsin deficiency Bronchiectasis Honeycombing seen in late stage scarring interstitial lung diseases

Gupta N et al, AJRCCM 2015

- Patogenesi
- Biomakers, diagnosi
- Terapia

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

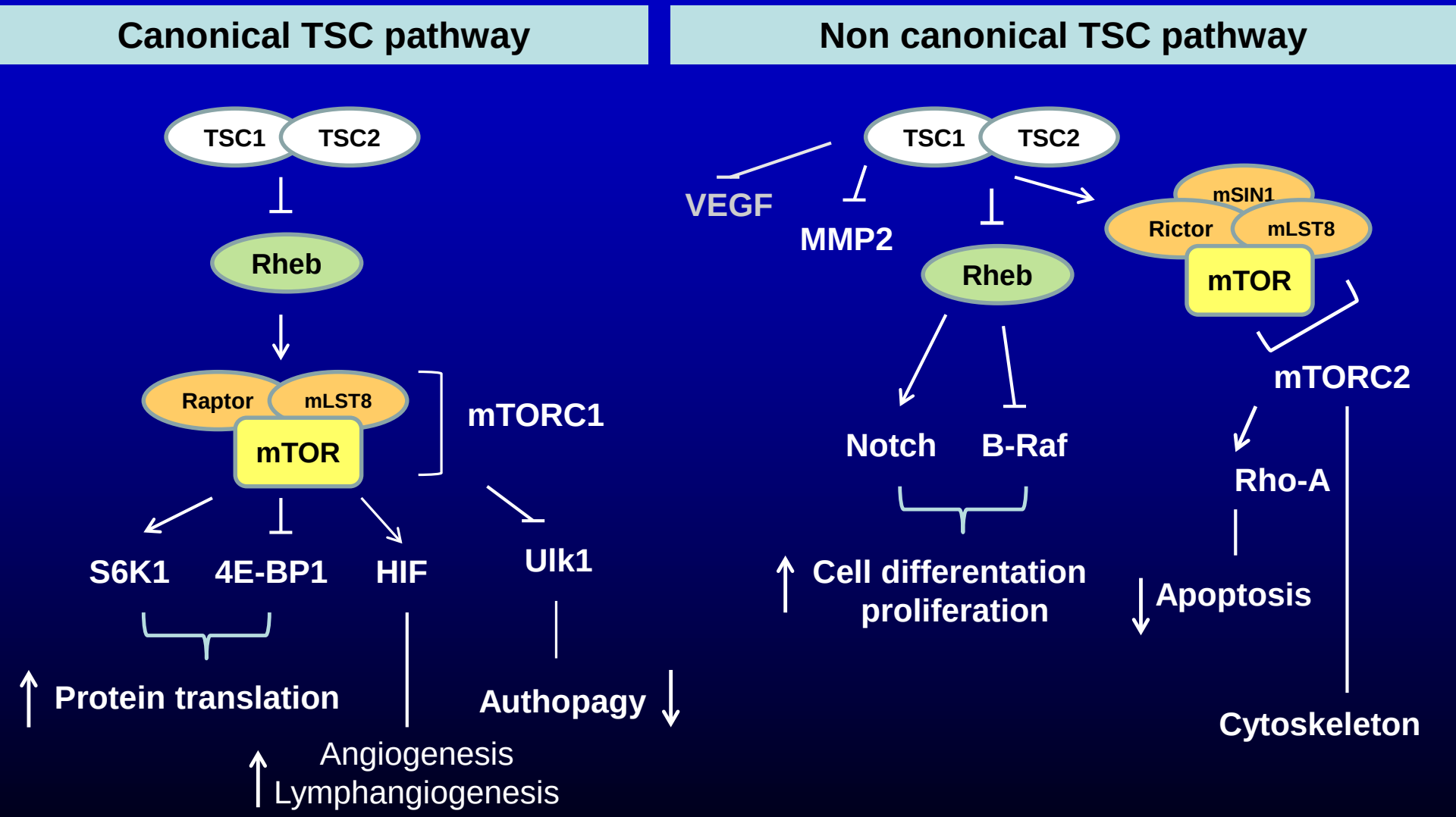
But the source of LAM cells is still unknown

(Uterus? Angiomyolipomas? Lymphatics?)

LAM cells show little evidence of proliferation, no atiphya

Henske1 EP and McCormack FX, J Clin Invest 2012
McCormack FX, et al. AJRCCM 2012

LAM - Phatogenesis



TSC loss of herozygosis (LOH) in cells from body fluids

Molecular and genetic analysis of disseminated neoplastic cells in lymphangioleiomyomatosis

Denise M. Crooks*, Gustavo Pacheco-Rodriguez*, Rosamma M. DeCastro*, J. Philip McCoy, Jr.†, Ji-an Wang‡, Fumiyuki Kumaki*, Thomas Darling‡, and Joel Moss*§

Crooks et al, PNAS 2004

Sirolimus Decreases Circulating Lymphangioleiomyomatosis Cells in Patients With Lymphangioleiomyomatosis

Xiong Cai, PhD; Gustavo Pacheco-Rodriguez, PhD; Mary Haughey, RN, BSN; Leigh Samsel, MS; Suowen Xu, PhD; Hai-Ping Wu, BS; J. Philip McCoy, PhD;

Cai et al, Chest 2014

- Link between primary LAM lesions and the process that facilitates dispersion of cells with metastatic potential

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

Phatogenesis: role of estrogen

- ✓ Female predominance
- ✓ Frequent occurrence during childbearing age
- ✓ Reported worsening following the administration of estrogens or during pregnancy
- ✓ Presence of estrogen receptors (ER) in LAM cells

Estrogen interacts with signaling events in LAM cells

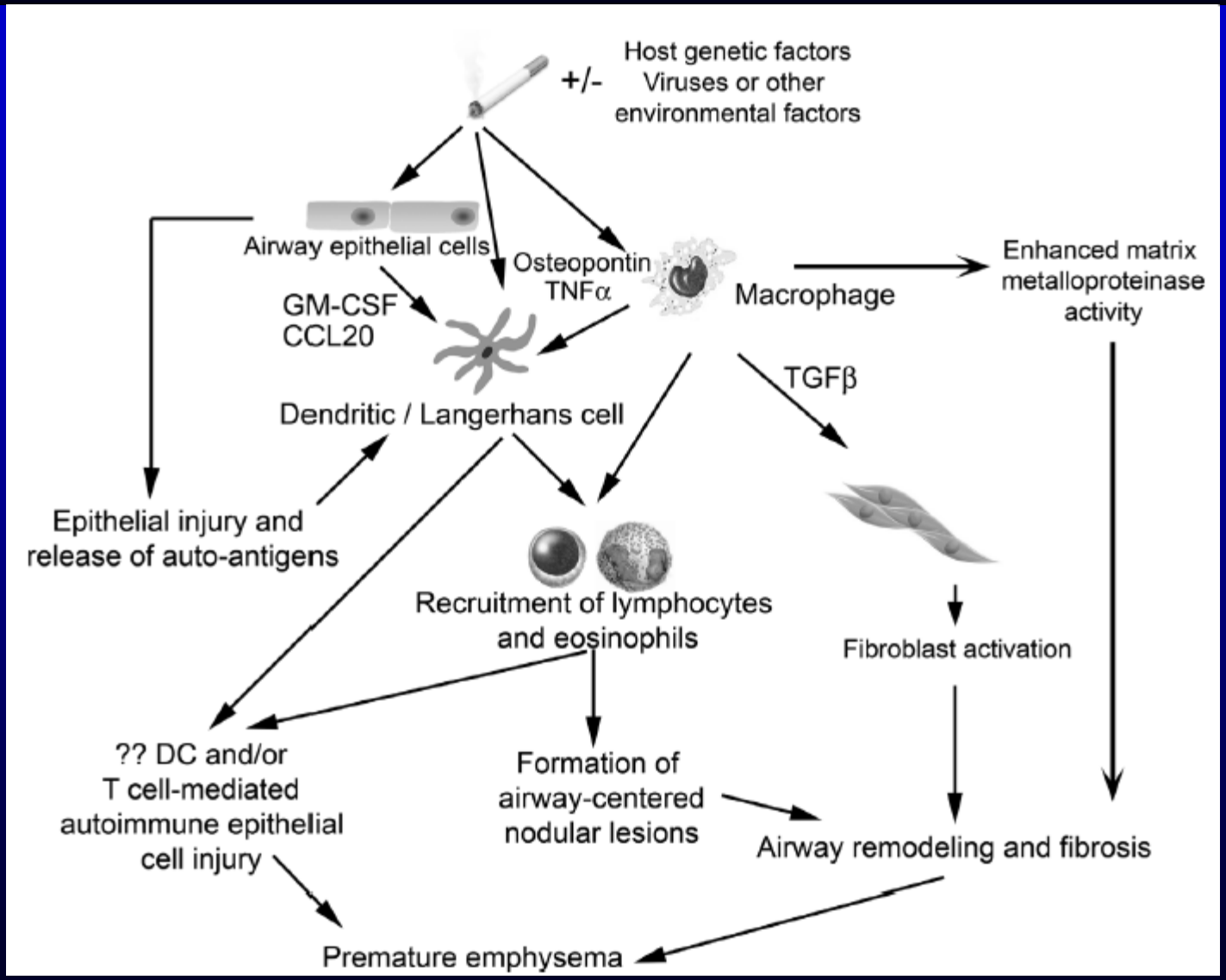
Cellular models

- to promote the proliferation of Tsc-null rat ELT3 leiomyoma-derived cells
- to stimulate the transcription of the late response-gene Fra1 associated with epithelial to-mesenchymal transition. –this effect is enhanced by TORC1/S6K signaling
- to increase MMP-2 activity

Animal models

- to stimulate growth of human AML TSC2+ cells
- to promote the survival and pulmonary metastasis of Tsc 2−/− ELT3 cells

PLCH - Pathogenesis



Suri HS et al, Orphanet Journal of Rare Diseases 2012

PLCH: a neoplastic or a reactive condition ?

Is PLCH a clonal proliferative process or a polyclonal reactive process induced by cigarette smoke ?

PLCH: a neoplastic or a reactive condition ?

The NEW ENGLAND JOURNAL of MEDICINE

Volume 331:154-160 July 21, 1994 Number 3

Langerhans'-Cell Histiocytosis (Histiocytosis X) --

A Clonal Proliferative Disease

Cheryl L. Willman, Lambert Busque, Barbara B. Griffith, Blaise E. Favara, Kenneth L. McClain, Marilyn H. Duncan, and D. Gary Gilliland

The American Journal of Surgical Pathology *25(5): 630–636, 2001*

Pulmonary Langerhans' Cell Histiocytosis

Molecular Analysis of Clonality

Samuel A. Yousem, M.D., Thomas V. Colby, M.D., Yuan-Yuan Chen, B.S., Wen-Gang Chen, B.S., and Lawrence M. Weiss, M.D.

Evidence of clonality in LCH

First evidence of BRAF mutations demonstrated clonality when LCH presents as a systemic disease or as a solitary mass

Badalian-Very G et al, Blood 2010
Kamionek M et al, *Mod Pathol* 2012
Satoh Tet al, *PLoS One* 2012

BRAF mutations in PLCH

- *B-Raf* is part of the intracellular Ras-Raf/MAPK signaling pathway that is responsible for several cell functions

  (cell proliferation, differentiation, migration, and senescence/apoptosis)

- Mutations in *BRAF* have been associated with the development of aggressive neoplasms

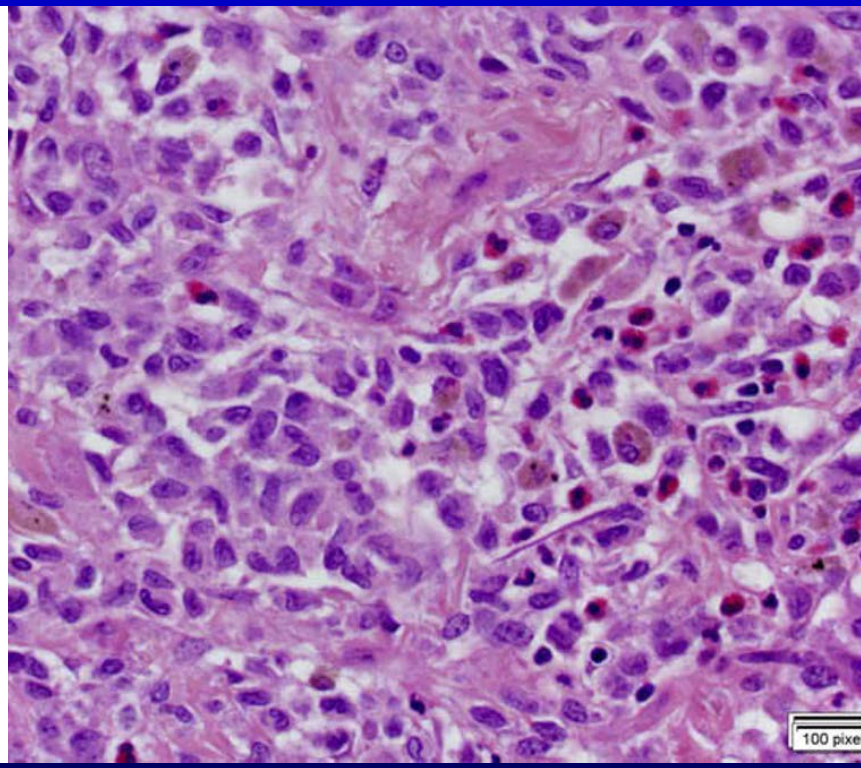
  (including malignant melanoma, colonic adenocarcinoma, papillary thyroid carcinoma, and lung adenocarcinoma).

Concordante occurrence of *BRAF* V600E mutations in PLCH

Table 1—*Clinicopathologic and Molecular Data on Cases of Pulmonary Langerhans Cell Histiocytosis*

Case No.	Age/Sex	Smoking (Active)	HRCT Scan Finding	Nodules With *BRAF* V600E Mutation	
				Positive	Negative
1	36/F	+	Bilateral nodules	5	0
2	50/F	+	Bilateral nodules	0	4
3	68/M	+	Bilateral nodules	0	2
4	65/M	+	Bilateral nodules	0	9
5	52/M	+	Bilateral nodules	2	0

F = female; HRCT = high-resolution CT; M = male.



Identical BRAF V600E mutation was identified in seven nodules from two cases
In other cases distinct nodules lacked any mutation, including *BRAF* V600E

Yousem SA, Chest 2013

BRAF V600E Expression in LCH

TABLE 1. Demographics of Patients With PLCH and Extrapulmonary LCH

	PLCH (n = 25)	Extrapulmonary LCH (n = 54)
Age (mean [± SD]) (y)	42.0 (11.4)	27.6 (21.8)
Sex, male (n [%])	10 (40.0)	37 (68.5)
Current or ex-smoker (n [%])	25 (100.0)	26 (48.1)
BRAF V600E IHC–positive cases		
n (%)	7 (28.0)	19 (35.2)
Age (mean [± SD])	45.3 (8.1)	27.6 (22.1)
Sex, male (n [%])	2 (28.6)	13 (68.4)
BRAF V600E IHC–negative cases		
n (%)	18 (72.0)	35 (64.8)
Age (mean [± SD])	40.7 (12.5)	26.9 (22.0)
Sex, male (n [%])	8 (44.4)	24 (68.5)

- 28% of PLCH cases were positive for BRAF V600E expression (immunohistochemistry)
- All but one cases were also positive by mutation analysis (PCR)
- In PLCH patients, the cumulative tobacco exposure at the time of diagnosis was significantly higher in BRAF V600E positive than in BRAF V600E negative cases

Roden AC, Am J Surg Pathol 2014

PLCH: a neoplastic or reactive condition ?

At least a proportion of PLCH is a cigarette smoke induced or promoted dendritic cell neoplasm that is associated with a prominent immune-inflammatory component

Gupta N et al, AMRCCM 2015

Mutations in PLCH

- BRAF mutations have been identified in up to 67% of cases of PLCH
- Identical but mutually exclusive MAPK/ ERK pathway mutations (BRAF, MAP2K1 or KRAS) were found supporting a neoplastic/clonal origin
- NRAS mutations were found in 40% of pulmonary lesions explaining the MAPK activation in non BRAF, non MAP2K1 mutated lesions

Chilosi M et al, Leuk Lymphoma 2014
Kamionek M et al, Histopathology 2016
Mourah S et al, ERJ 2016

- Patogenesi
- Biomakers, diagnosi
- Terapia

LAM – Biomarkers
VEGF-D

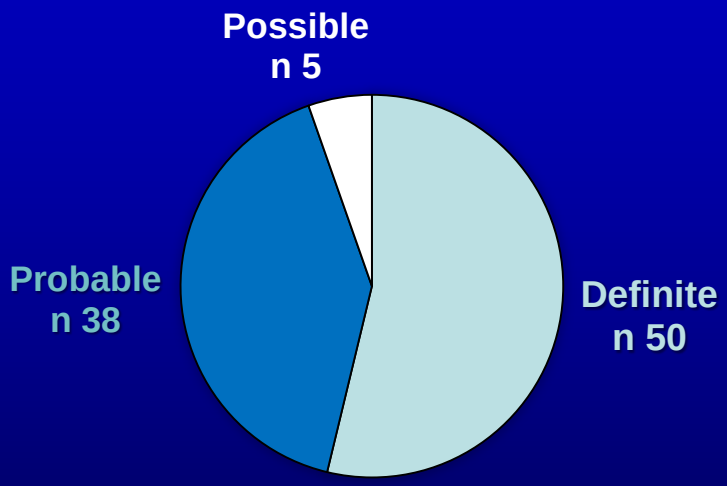
2006	Seyama K et al.	VEGF-D is increased in serum of patients with LAM
2008	Young et al.	VEGF-D serum levels are higher in LAM than in similar cystic or chylous lung diseases
2010	Young et al.	VEGF-D level higher than 800 pg/mL in a woman with typical changes on high-resolution CT scan is diagnostically specific for LAM, and identifies LAM in women with TSC
2010-	VEGF-D serum level has been used as diagnostic criteria in MILES trial and in *RAD001X2201* trial	
2014	**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** (Young LR et al, Lancet Respir Med 2013)	

VEGF-D

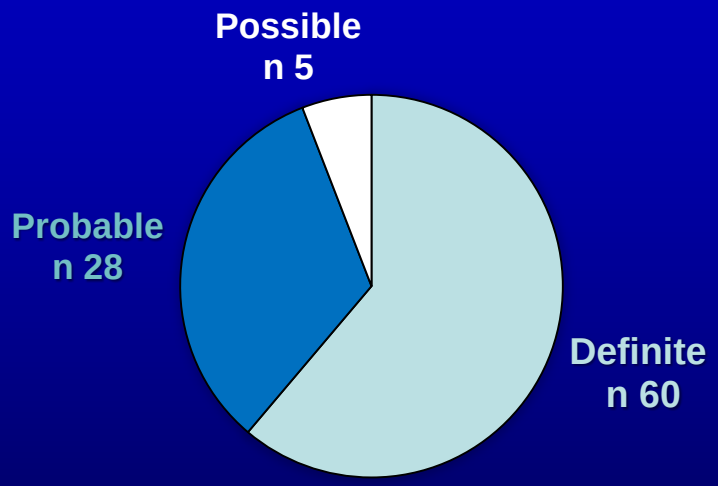
Ospedale San Giuseppe experience

137 patients, 44 with biopsy

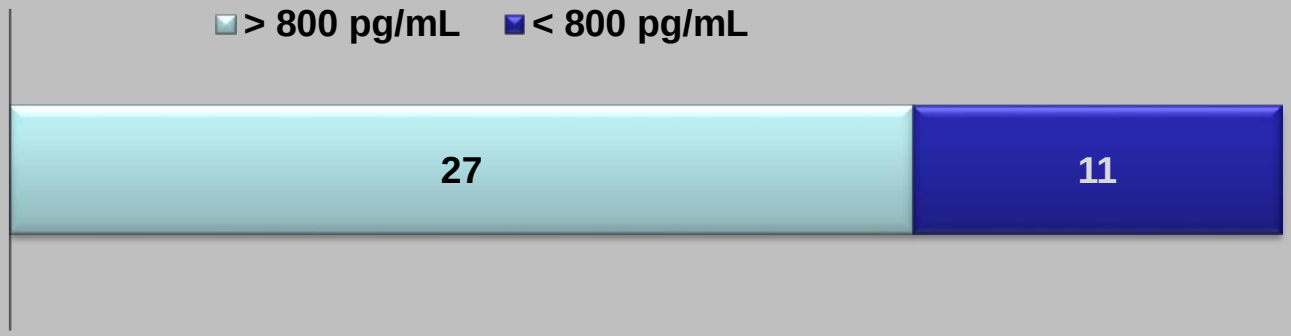
GUIDELINES CRITERIA



BEYOND GUIDELINES (VEGFD)

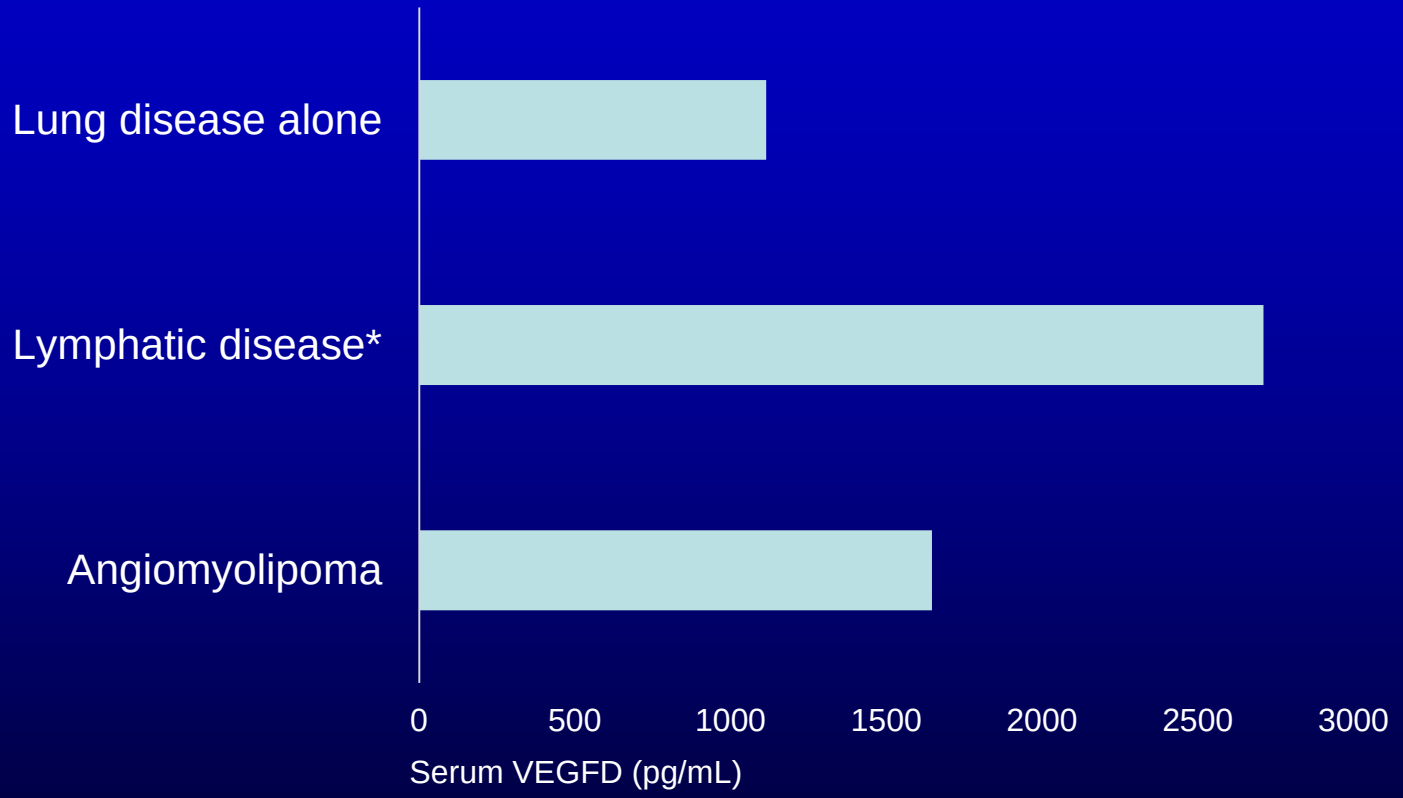


VEGFD levels in 38 pts with definite LAM based on ERS guidelines



VEGF-D

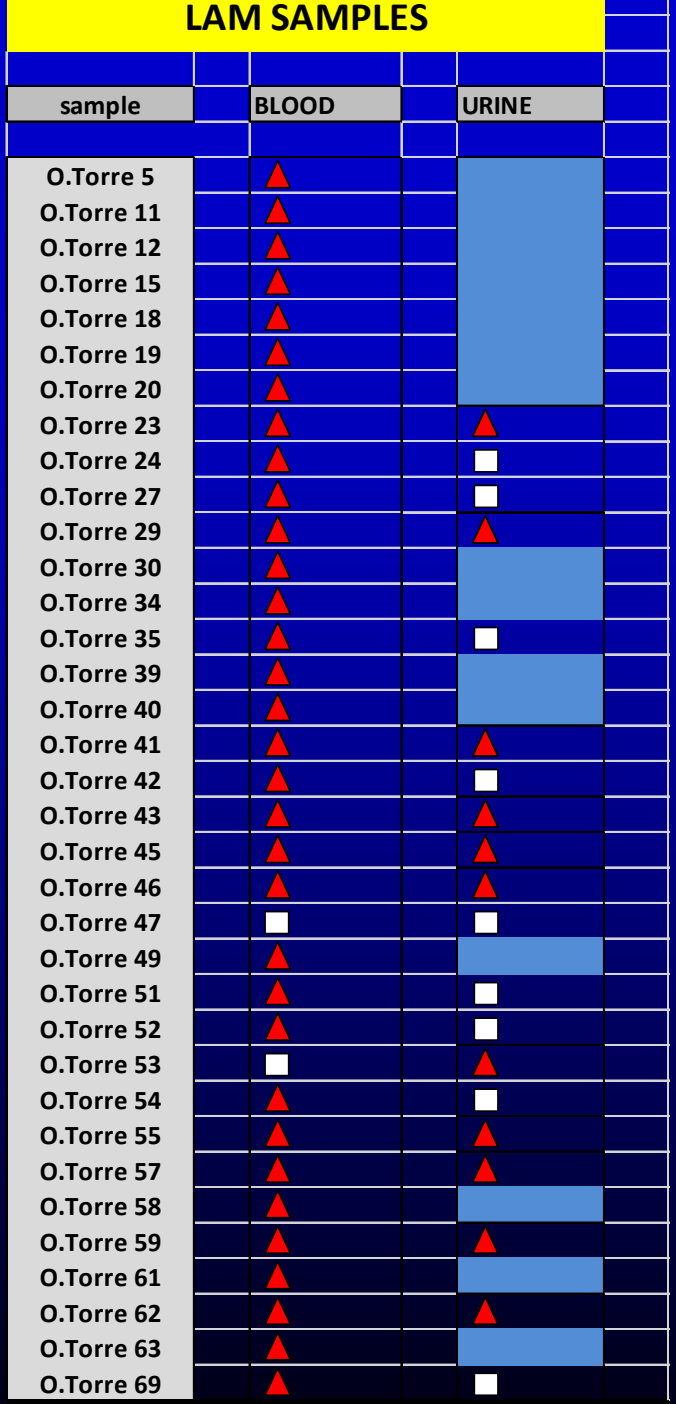
Ospedale San Giuseppe experience



* Chilous effusions, lymph nodes, lymphangioleiomyomas

LAM - Biomarkers

- Serum and/or urinary levels of MMPs
  - LAM nodules have been shown to contain MMP activators and inhibitors
  - Serum and urinary levels of MMP-9 have been found to be higher in patients with LAM than in normal subjects
- TSC loss of herozygosis (LOH) in cells from body fluids
  - LAM cells, identified by TSC2 LOH, have been isolated from the blood and other body fluids of LAM patients and they are no longer decectible after treatment with sirolimus
- Proteins involved in extracellular matrix remodelling?
  - Proteins involved in extracellular matrix remodelling are differentially expressed in LAM serum compared to control serum



TSC LOH

Ospedale San Giuseppe experience

▲ LOH

□ ROH

Blood (CD45-/glicoforina-, CD45-/glicoforina+): 94% (33/35)

Urine (CD44+/CD9+, CD44+/CD9-): 55%(11/20)

BAL in PLCH

- High specificity (CD1a>5%) but low sensitivity
- In an appropriate clinical context BAL can be used to establish the diagnosis of PLCH
- In patients with atypical clinical and/or radiological presentation it can be used to role out interstitial lung diseases with more typical lavage findings (e.g. sarcoidosis) and pulmonary infections (excavated forms of *Pneumocystis Jiroveci* pneumonia or mycobacterial infections)

Tazi A et al, Eur respir J 2006
Harari et al, Respir Med. 2012
Baqir M eta al, Bronchology Interv Pulmonol 2013

- Patogenesi
- Biomakers, diagnosi
- Terapia

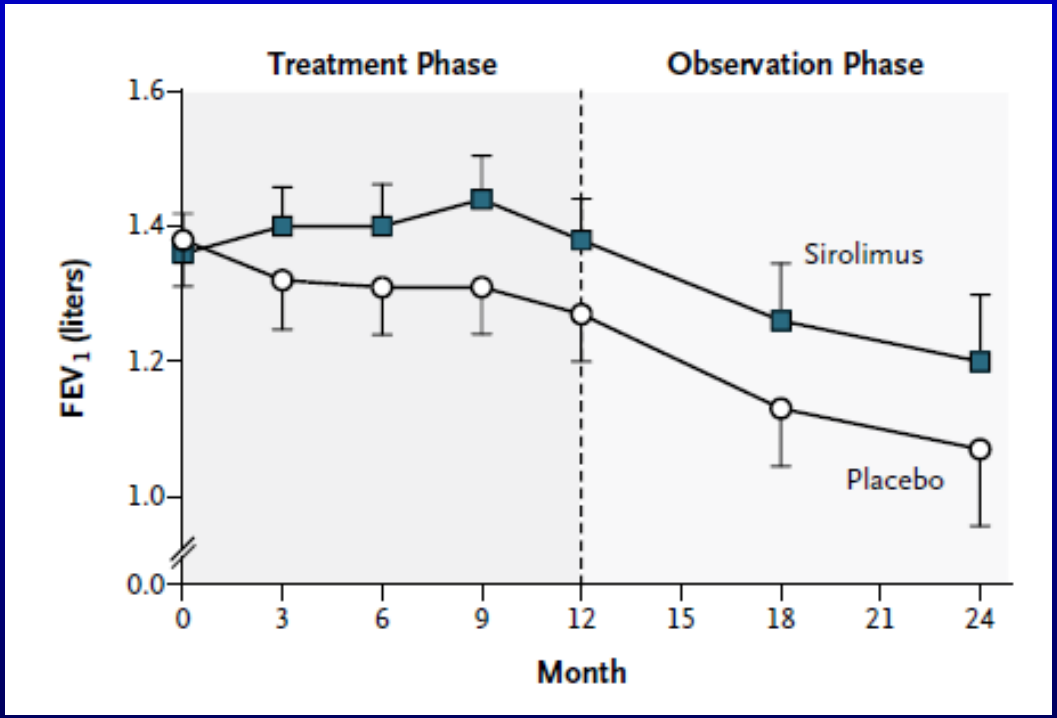
LAM - Treatment

mTOR inhibitors

2011	**MILES** (Sirolimus)	Randomised, double-blind, placebo-controlled	Efficacy and safety for sirolimus in LAM
2011	**TESSTAL** (Sirolimus)	Non-randomized, open label trial	Efficacy and Safety rapy for renal angiomyolipmoas in TSC-LAM and S-LAM
2013	**EXIST-1** (Everolimus)	Randomised, double-blind, placebo-controlled	Efficacy and safety in subependymal giant cell astrocytomas
2013	**EXIST -2** (Everolimus)	Randomised, double-blind, placebo-controlled	Angiomyolipoma response in TSC or S-LAM
2015	**RAD001X2201** (Everolimus)	Open-label, within-patient multiple dose escalation in LAM	Efficacy and safety for everolimus in LAM

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, NEJM 2011

Beyond the MILES trial

- Sirolimus is effective on lymphatic manifestations of LAM (chylous effusions, size of lymphangioleiomyomas)

Taveira-Dasilva AM, Ann Intern Med. 2011

- Treatment with sirolimus for a period of about 3.5 years stabilized lung function (decline in FEV1 and DLCO), and changes in lung volume occupied by cysts with an acceptable safety profile

Yao J, AJRCCM 2014

- A retrospective study of 15 pts with low dose sirolimus (serum levels <5 ng/mL) showed stabilization of lung function and resolution of chylothorax

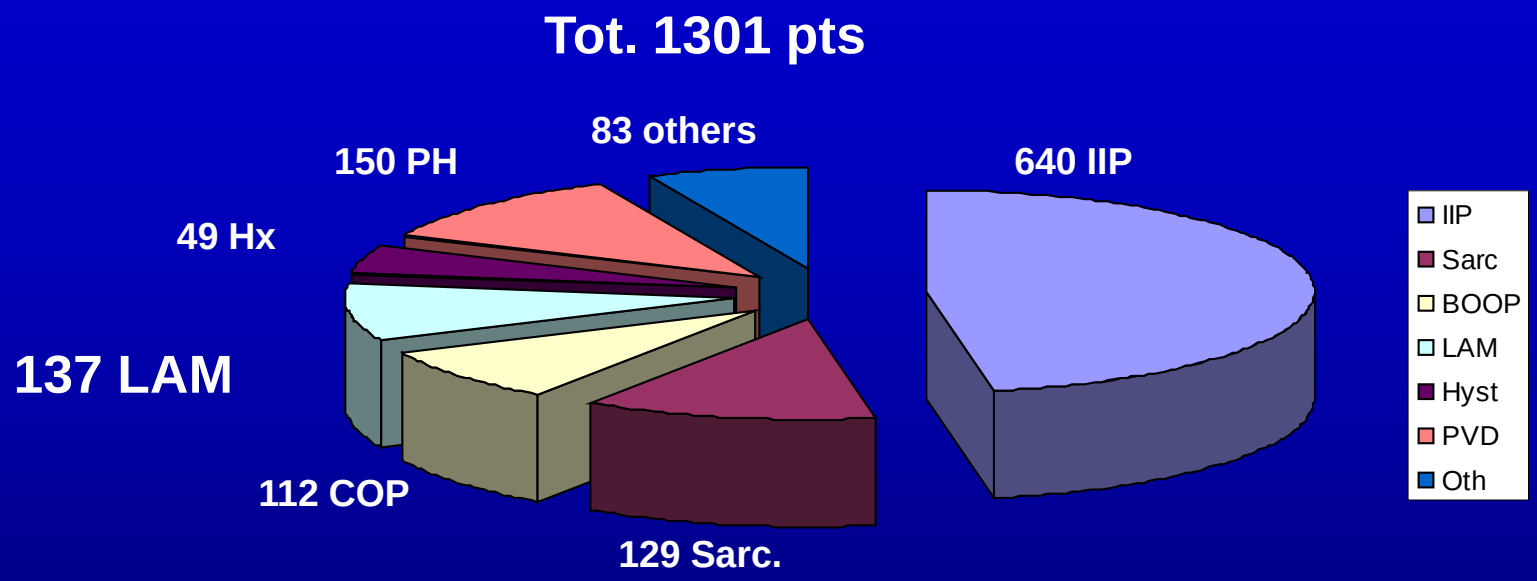
Ando K, Respir Invest 2013

Sirolimus: current indications

- Patients with abnormal lung function
- Asymptomatic patients who are declining rapidly
- Symptomatic patients
- Problematic chylous effusions and lymphangioleiomiomas

Rare Lung Diseases

Ospedale San Giuseppe Experience (1999- 2016)



Mean age at diagnosis: 36 years
24 TSC-LAM

54 pts treated with Sirolimus, 37 pts with > 1 year follow-up

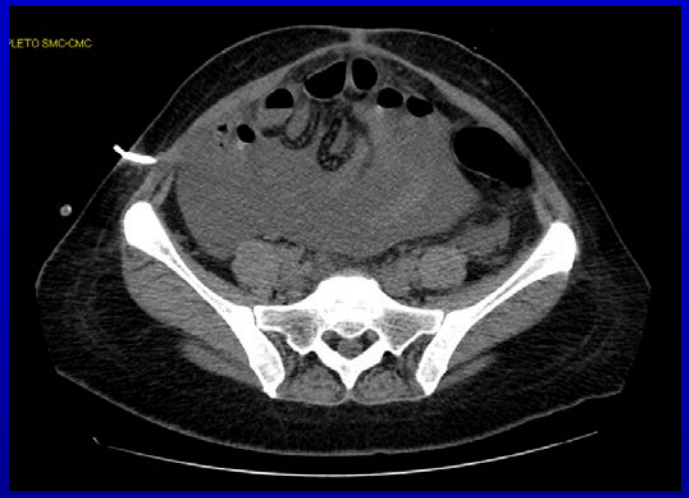
- mean FEV1 decrease 119 ml/year in pre-treatment period
- mean FEV1 increase 62 ml/year in treatment period

4 pts showed declining lung function after two year treatment period

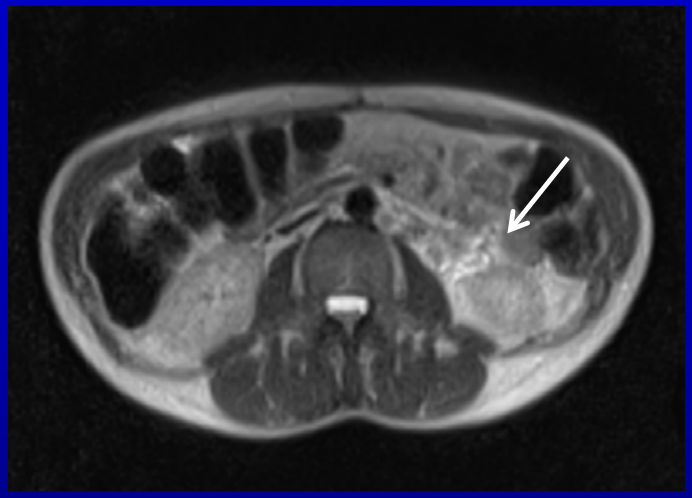
4 pts discontinued the therapy because of adverse events

Effectiveness of sirolimus in LAM

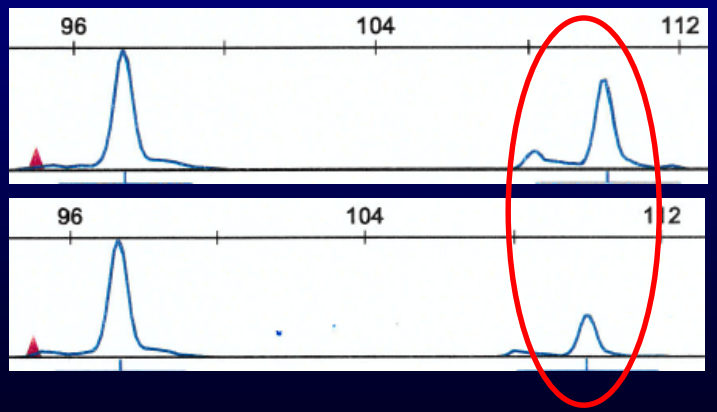
Before sirolimus

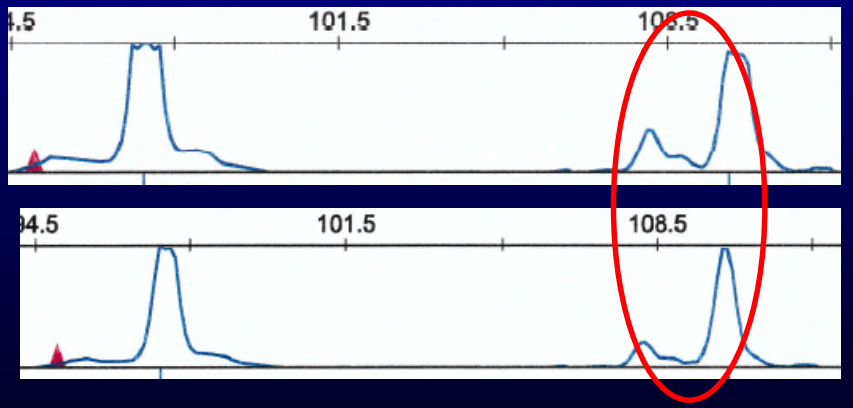


After 3 months of sirolimus



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

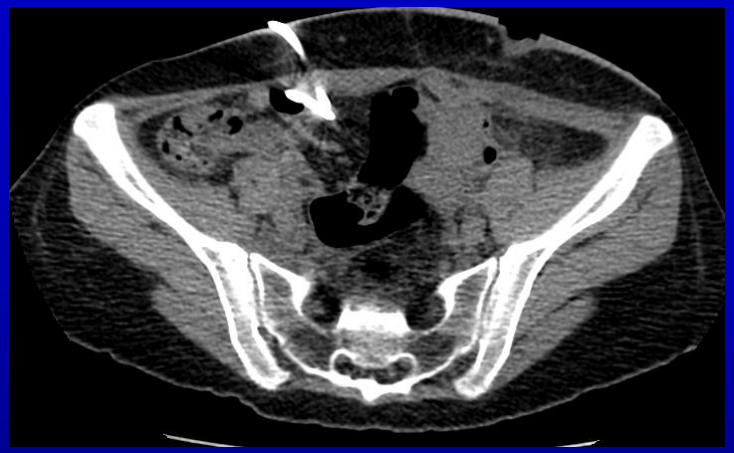




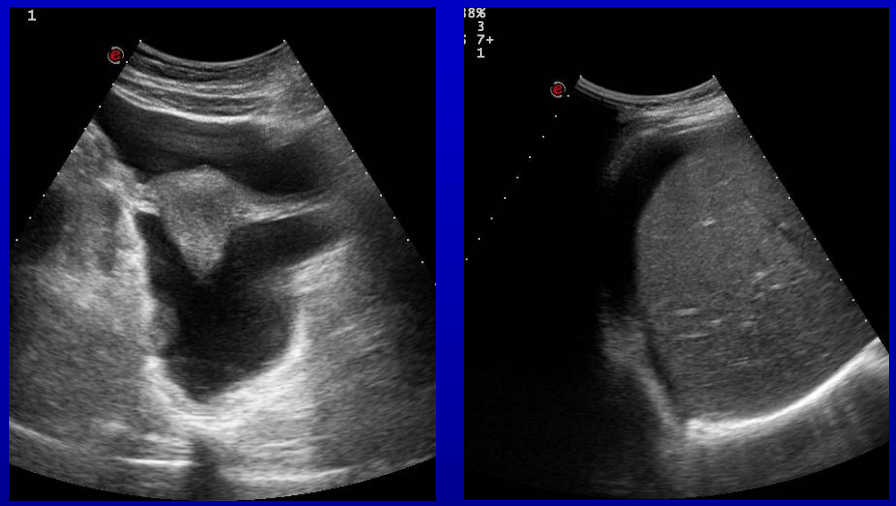
Elia D et al, Chest in press

Ineffectiveness of sirolimus in LAM

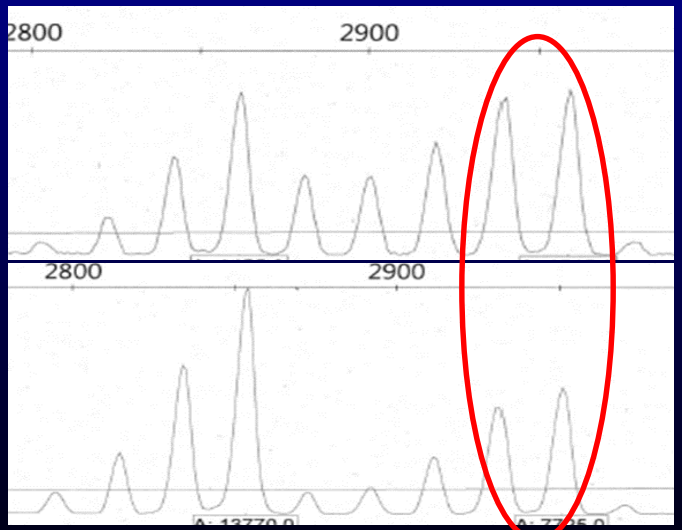
Before sirolimus

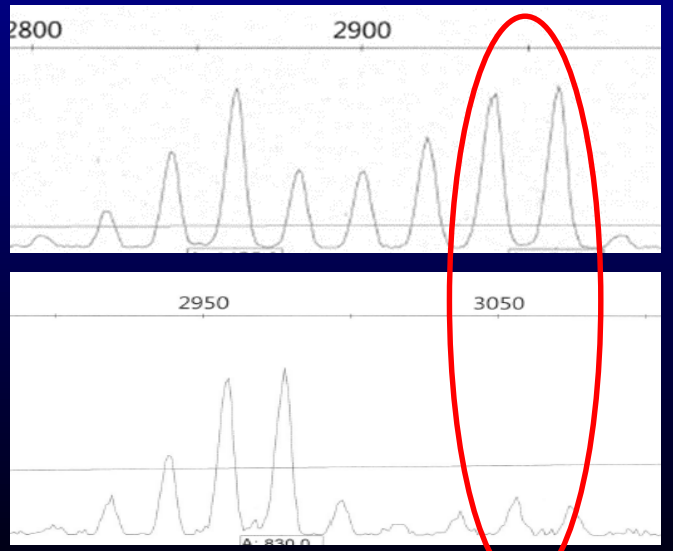


After 3 months of sirolimus



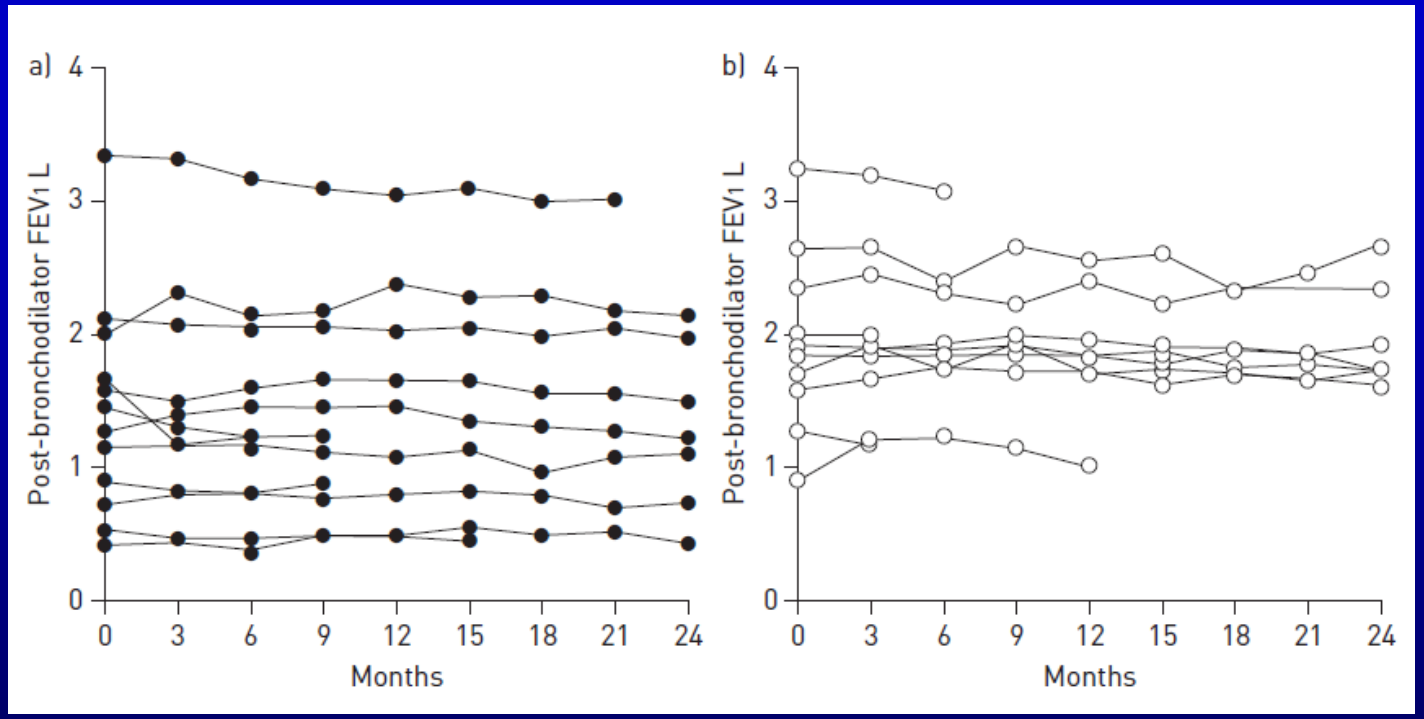
Serum VEGF-D: 776 pg/mL → 725 pg/mL





Doxycycline

A 2-year randomised placebo-controlled trial



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

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

Chang W, ERJ 2014

Treatment: the future

Cell-autonomous therapeutic approaches

Canonical and non-canonical TSC pathways

- Combination of mTOR and Autophagy inhibition:
  A trial of Sirolimus + hydroxychloroquine is ongoing
- Combination of mTOR inhibition and statins:
  A trial of Sirolimus and simvastin is ongoing
- Kinase inhibitors

Non cell-autonomous therapeutic approaches

- Inhibition of MMPs and other proteases
- Estrogen antagonism
- Inhibition of LAM cells utilizing melanocyte antigens

Treatment: the future

A pilot study of nintedanib for lymphangioleiomyomatosis

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

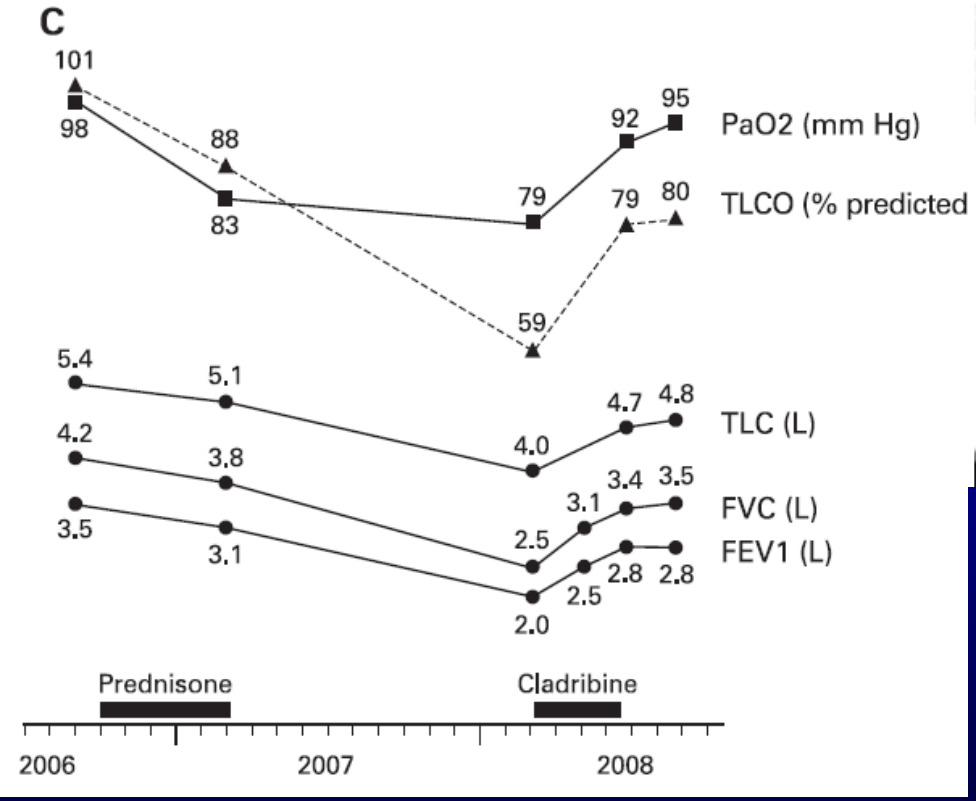
PLCH - TERAPIA

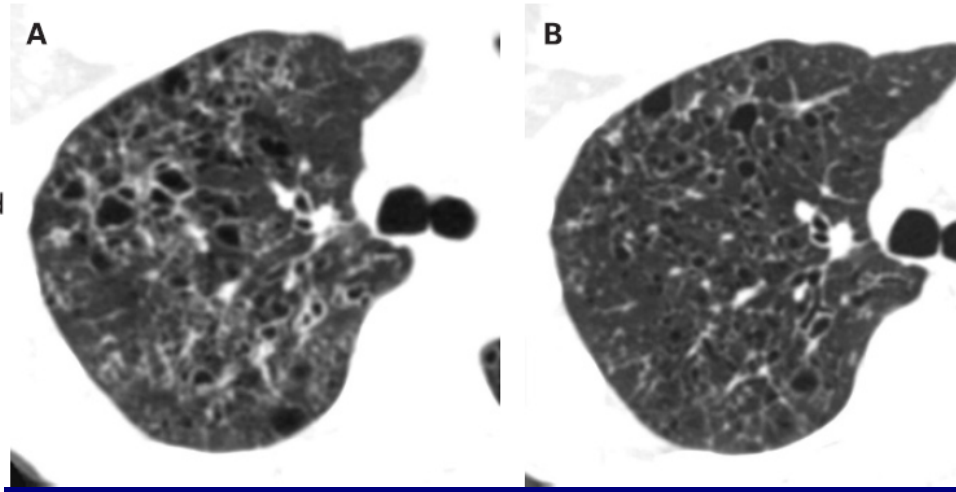
Smoking cessation is mandatory!

- No prospective or randomized trials about steroids
- Chemotherapeutic have been used in patients with progressive disease or in those with multiorgan involvement.

- Case reports and retrospective studies showed improvement of hemodynamic parameters in patients with PH treated with PH-therapies

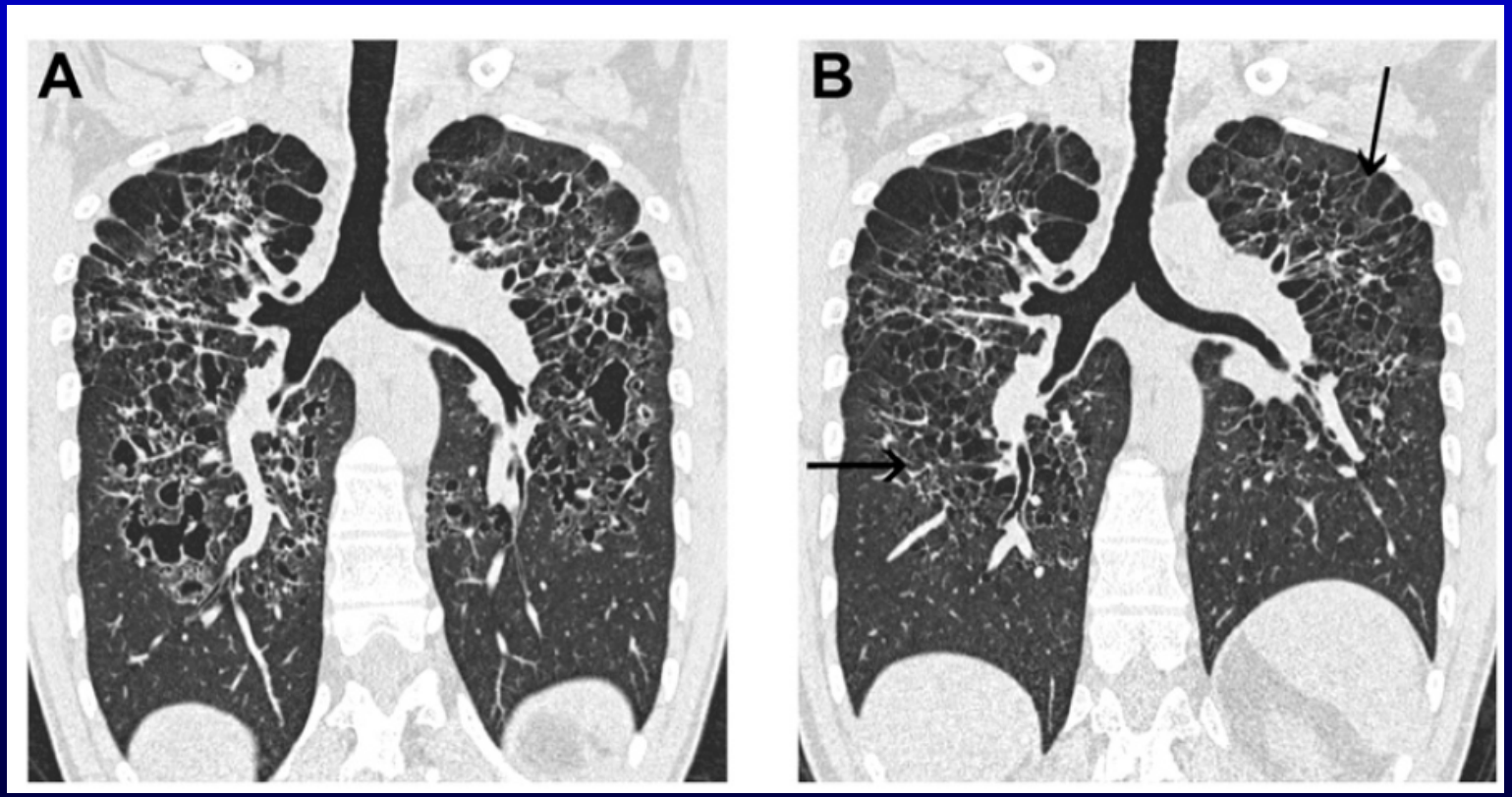
Progressive diffuse pulmonary Langerhans cell histiocytosis improved by cladribine chemotherapy (A case report)





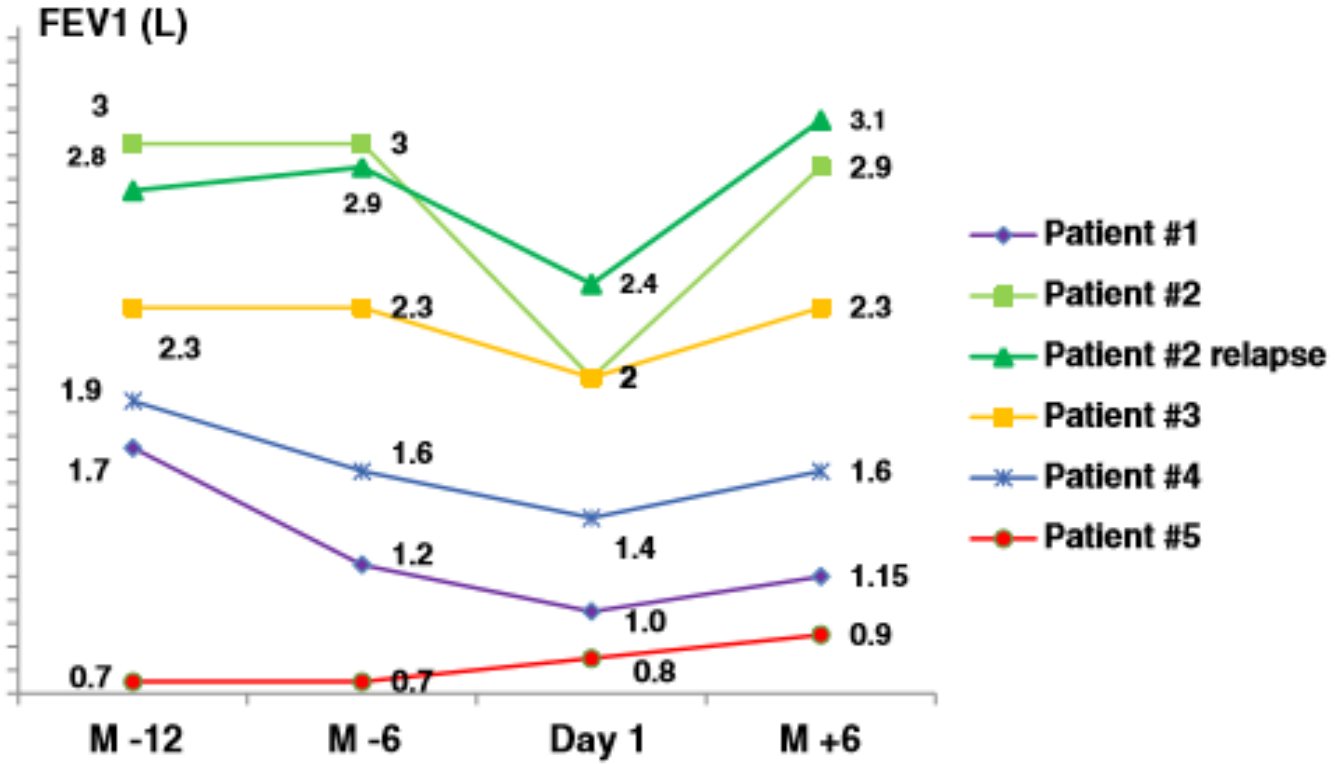
Lazor R et al, Thorax 2009

Cladribine Is Effective against Cystic Pulmonary Langerhans Cell Histiocytosis (case reports)



Lorillon G et al, AJRCCM 2012

Effectiveness of cladribine therapy in patients with PLCH (a retrospective study)



Evolution of forced expiratory volume in 1 second (FEV1) before and after cladribine therapy in 5 pts

M-12: 12 months before cladribine; M-6: 6 months before cladribine
Day 1 (cladribine): initiation of cladribine therapy; M + 6: 6 months after cladribine treatment.

Grobost et al, Orphanet Journal of Rare Diseases 2014

Cladribine in PLCH

Cladribine (2-chlorodeoxyadenosine) is a chemotherapeutic agent cytotoxic for lymphocyte and monocyte cells

Evaluation of Efficacy and Tolerance of Cladribine in Symptomatic Pulmonary Langerhans Cell Histiocytosis and Impairment of Lung Function (ECLA) trial is ongoing (Phase 2, Open Label)

PLCH: future issues

- Cladribine
- BRAF inhibitors?

Conclusions

Although a variable and sometime indolent or slowly progressive course, LAM e PLCH are neoplastic diseases

Biomarkers are available for diagnosis and follow-up

Still finding new biomarkers is a main issue

Sirolimus is an effective and relatively safe treatment for LAM, but unresponsive or intolerant patients are not rare

New therapeutic approaches are needed

Cladribine may be an effective treatment for PLCH

Centro LAM e TSC dell'adulto

Ospedale San Giuseppe

Team multidisciplinare

- Pneumologia
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  - Dott.ssa Torre Olga
  - Dott. Cassandro Roberto
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- Neurologia
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- Cardiologia
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Centro LAM e TSC dell'adulto

Ospedale San Giuseppe

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- NIH
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