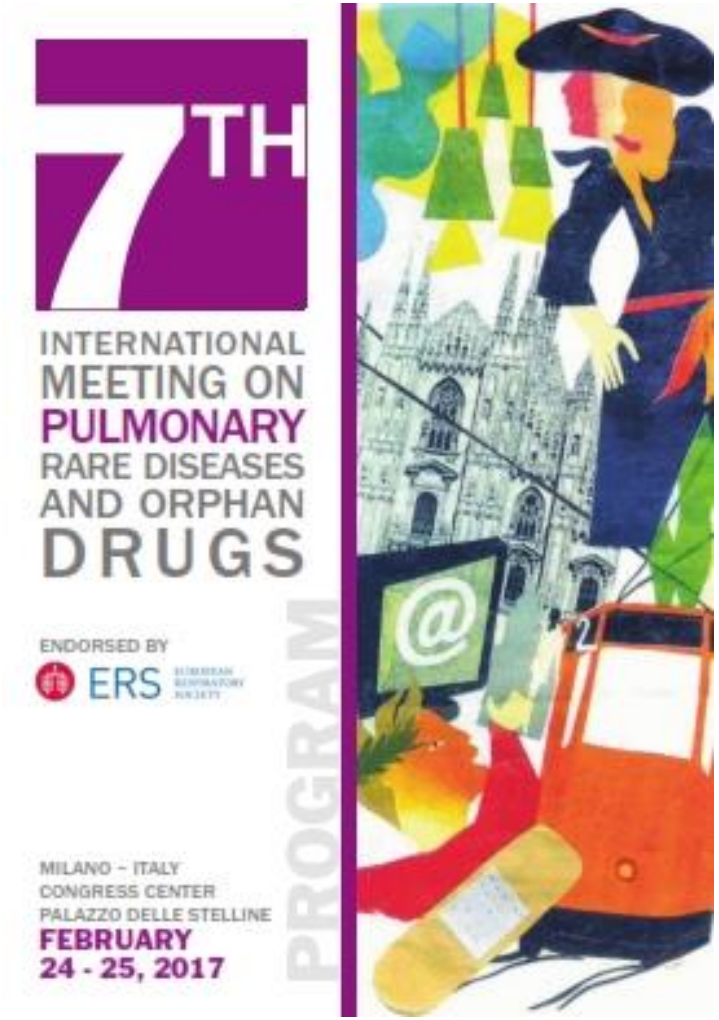


New insights in LAM and PLCH

Olga Torre
U.O. di Pneumologia e UTIR
Servizio di Emodinamica e Fisiopatologia Respiratoria
Ospedale San Giuseppe - Milano

7° th International Congress on Rare Pulmonary Diseases and Orphan Drugs

Milan, February 24-25, 2017

New and old questions

Few answers

New and old questions

1. What are LAM and PLCH?

2. Are there useful biomarkers for diagnosis and follow-up?

3. Do we have a therapy?

1. What are LAM and PLCH?

2. Are there useful biomarkers for diagnosis and follow-up?

3. Do we have a therapy?

Classification of Diffuse Cystic Lung Diseases

Gupta N et al, AJRCCM 2015

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

Is 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

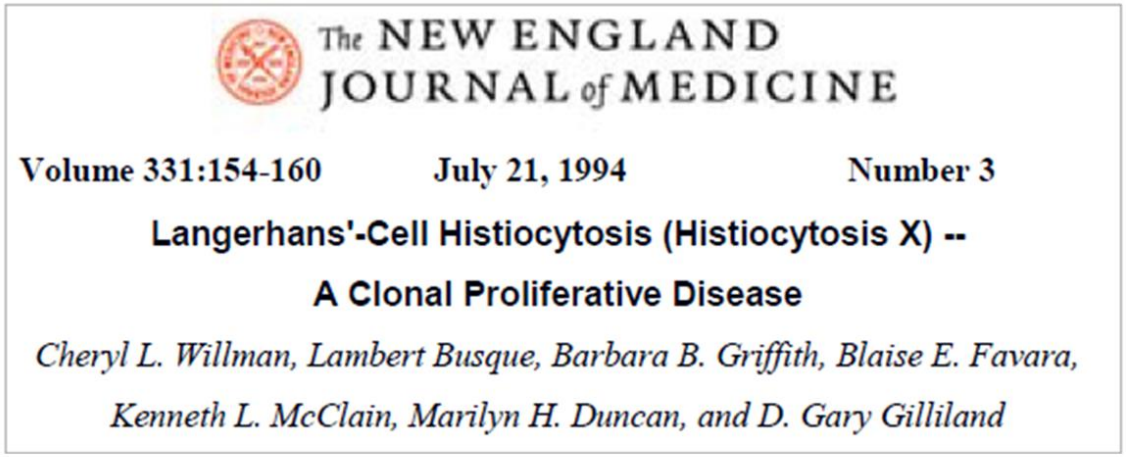
(Uterus? Kidneys? Lymphatics?)

LAM cells show no atypia

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

PLCH: a neoplastic or a reactive condition ?

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



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 PLCH: *BRAF* mutations

Pulmonary Langerhans Cell Histiocytosis

Profiling of Multifocal Tumors Using Next-Generation Sequencing Identifies Concordant Occurrence of *BRAF* V600E Mutations

Samuel A. Yousem, MD, FCCP; Sanja Dacic, MD, PhD; Yuri E. Nikiforov, MD, PhD; and Marina Nikiforova, MD

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

Yousem SA et al, Chest 2013

ORIGINAL ARTICLE

BRAF V600E Expression in Langerhans Cell Histiocytosis

Clinical and Immunohistochemical Study on 25 Pulmonary and 54 Extrapulmonary Cases

28% of PLCH cases were positive for BRAF V600E expression

Roden AC et al, Am J Surg Pathol 2014

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 PLCH

- BRAF mutations have been identified in up to 67% of cases of PLCH

- Identical but mutually exclusive MAPK/ ERK pathway mutations (BRAF, MAP2K1) were found supporting a neoplastic/clonal origin

Chilosi M et al, Leuk Lymphoma 2014
Kamionek M et al, Histopathology 2016

- NRAS mutations have been found

BRAF and NRAS mutations can be present in different areas within the same lung lesion supporting a polyclonal nature of LCs

Mourah S et al, ERJ 2016

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, AJRCCM 2015

Role of smoking

- Smoking induces accumulation of CD1a+ cells in the lungs
- Smoking stimulates local production of cytokines and osteopontin, which play a role in the recruitment, differentiation and activation of dendritic cells

1. What are LAM and PLCH?

2. Are there useful biomarkers for diagnosis and follow-up?

3. Do we have a therapy?

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

AMERICAN THORACIC SOCIETY DOCUMENTS

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

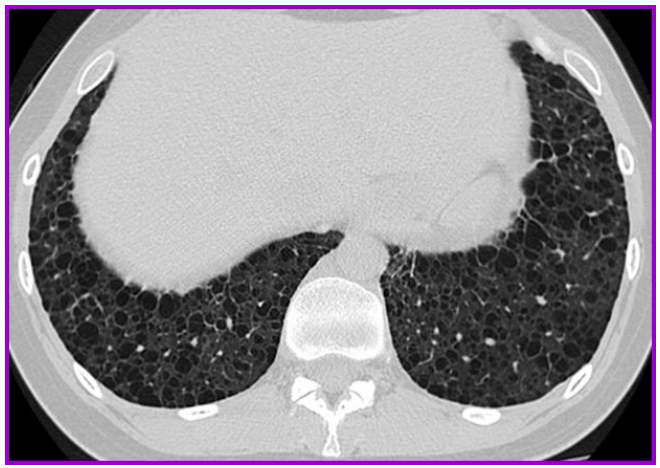
Francis X. McCormack, Nishant Gupta, Geraldine R. Finlay, Lisa R. Young, Angelo M. Taveira-DaSilva, Connie G. Glasgow, Wendy K. Steagall, Simon R. Johnson, Steven A. Sahn, Jay H. Ryu, Charlie Strange, Kuniaki Seyama, Eugene J. Sullivan, Robert M. Kotloff, Gregory P. Downey, Jeffrey T. Chapman, MeiLan 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



Characteristic lung HRCT

Numerous thin-walled lung cysts distributed diffusely throughout the lungs without sparing of lung bases

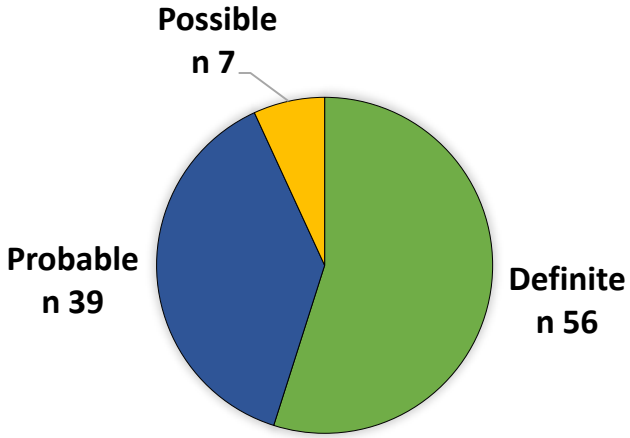
Johnson SR et al, ERJ 2010
McCormack FX et al, AJRCCM 2016

VEGF-D

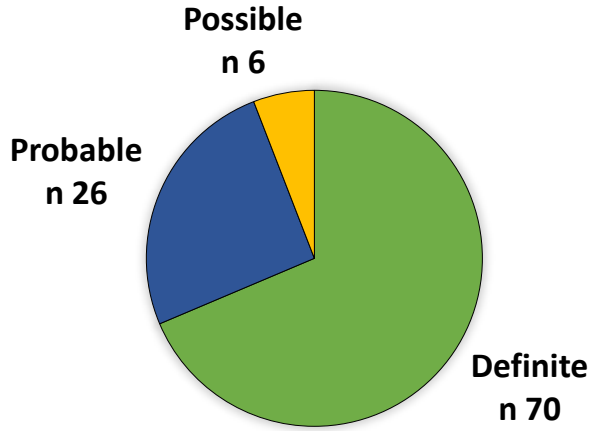
Ospedale San Giuseppe

Tot 152 patients, 50 with biopsy

102 patients

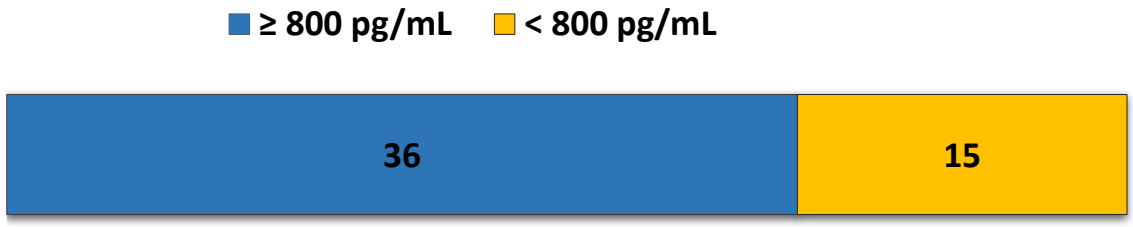


ERS 2010



ATS/JRS 2016

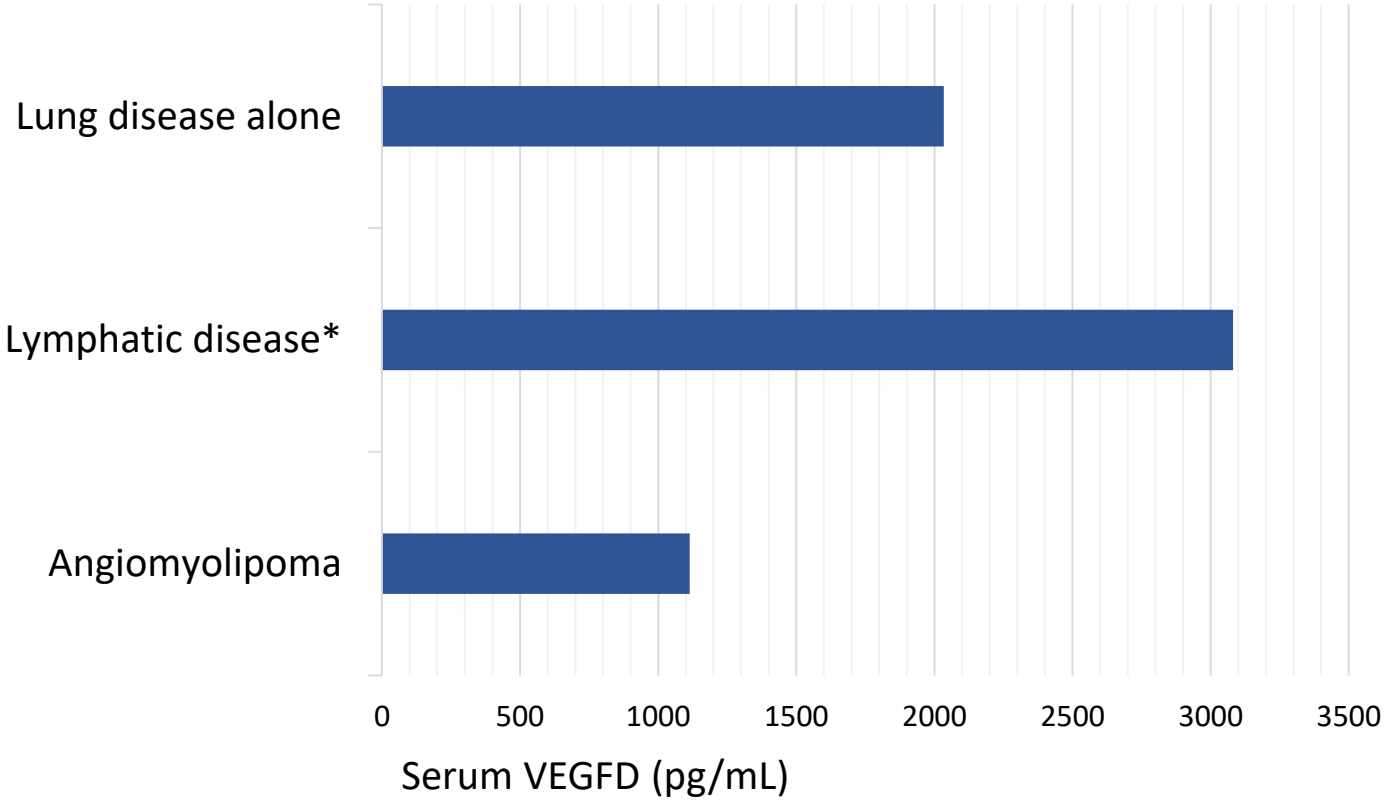
VEGFD is avalaible in 51 patients with definite LAM according to ERS Guidelines or biopsy



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

VEGF-D

Ospedale San Giuseppe

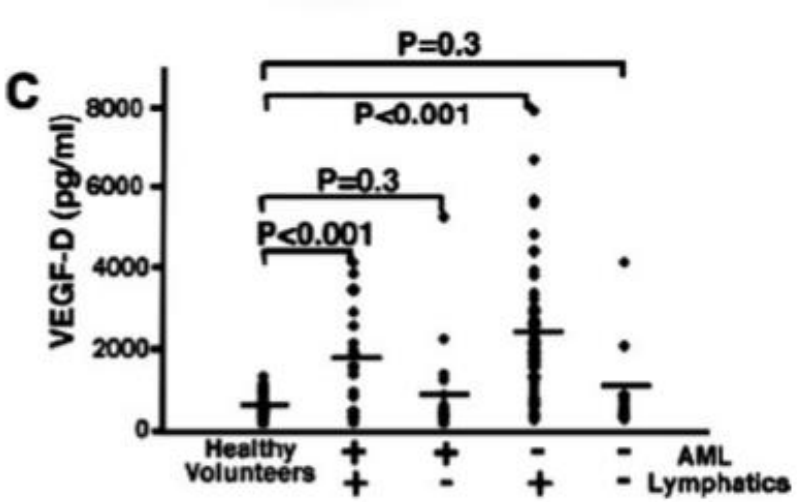


* Chylous effusions, lymph nodes, lymphangioleiomyomas

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

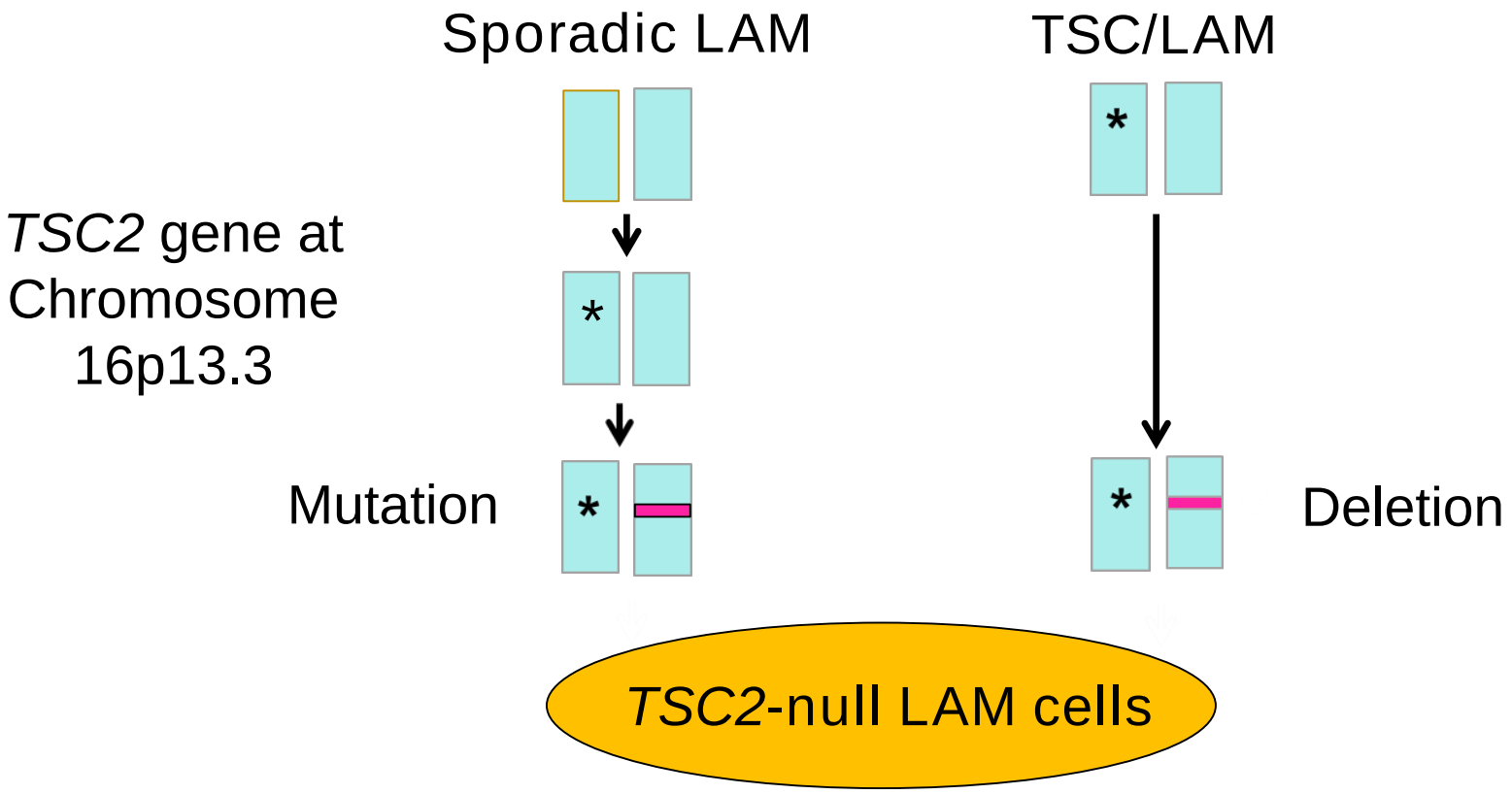
  - TSC LOH is involved in LAM pathogenesis

  - 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

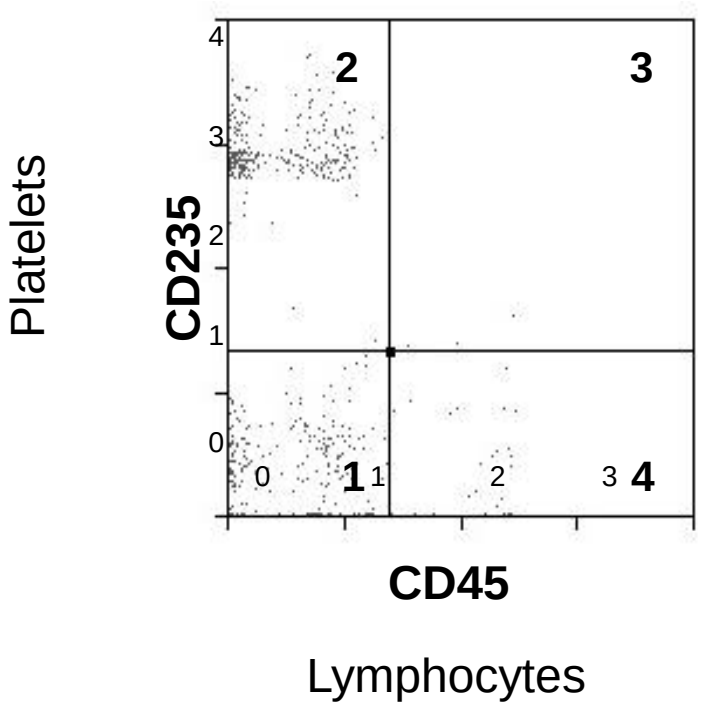
Crooks et al, PNAS 2004
Cai et al, Chest 2014

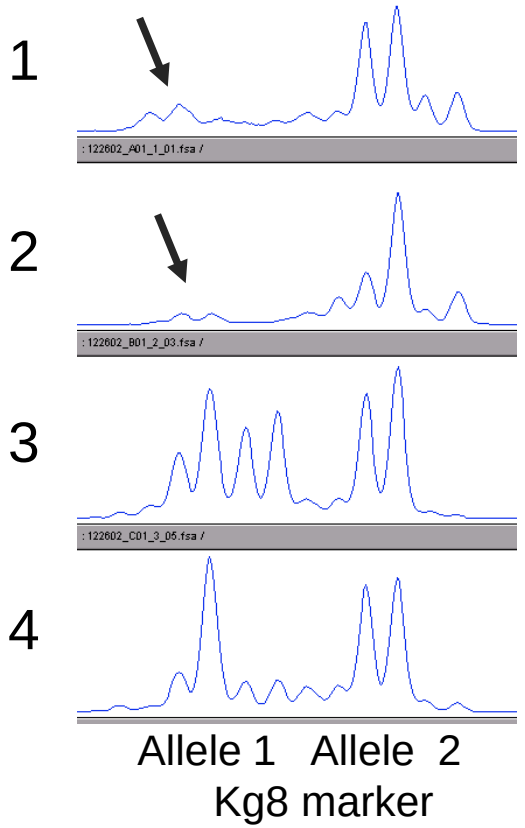
TSC2 Loss of Heterozygosity (LOH) and Knudson's Two-hit Model of Oncogenesis



Courtesy of Prof. Joel Moss

FACS and Chromosomal Analysis of Cells Isolated by Density Gradient Centrifugation





Different LOH patterns were found in different subpopulations of LAM cells, in different blody fluids, and over time in the same patient suggesting that a single patient may have different clones of LAM cells.

Courtesy of Prof. Joel Moss
Steagall WK et al, AJRCCM 2016

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	▲	□
O.Torre 53	▲	□
O.Torre 54	▲	□
O.Torre 55	▲	□
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)

TSC LOH has been reported in other pulmonary diseases but not in the same populations in which LAM cells are tipically found

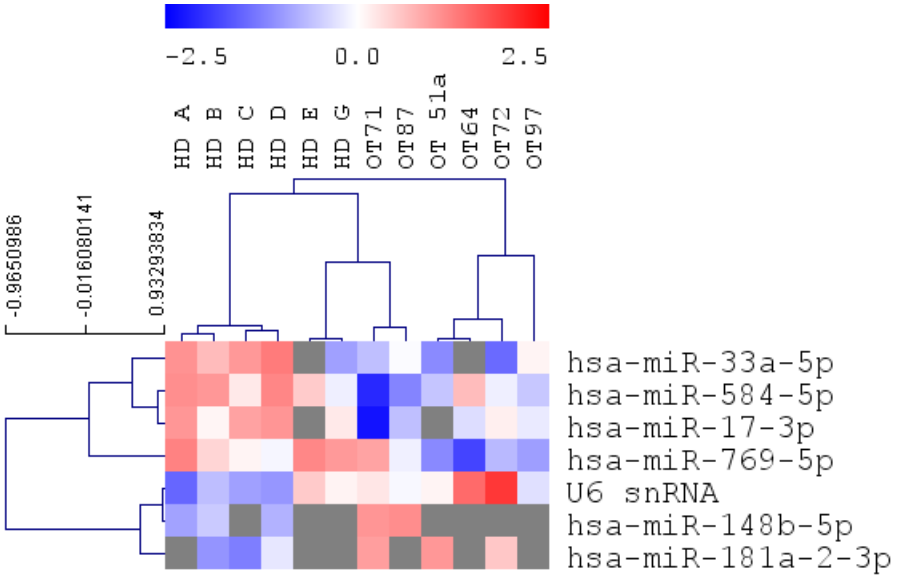
Zhang L et al, AJRCCM 2015

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



7 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

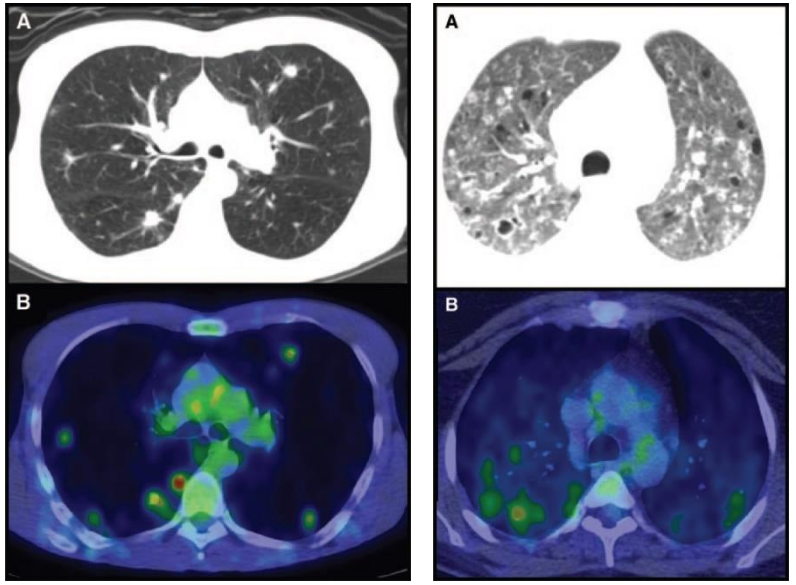
Torre O et al, unpublished

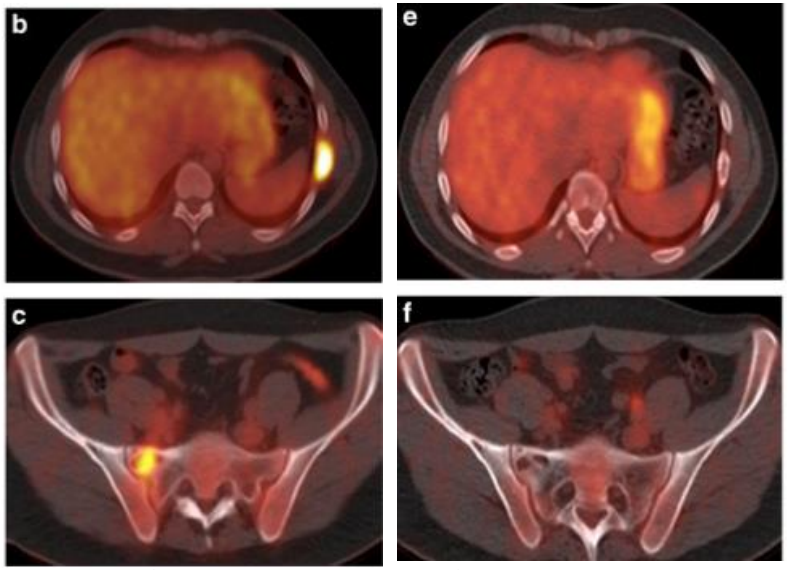
FDG-PET in PLCH

A retrospective study of 11 adults with PLCH

- Pulmonary nodules can be hypermetabolic, in which case they cannot be distinguished from malignant disease (mean max SUV >3)
- Thick-walled cysts can sometimes demonstrate high metabolic uptake

Krajicek et al, Chest 2009





Before After treatment

A retrospective study of 14 adults with multisystem LCH

- PET-CT showed additional lesions, mostly localized in bones
- PET-CT was very useful for the assessment of the spontaneous outcome and response to treatment
- PET-CT was only slightly helpful for the management of pulmonary involvement

Obert J et al, EJNMMI 2016

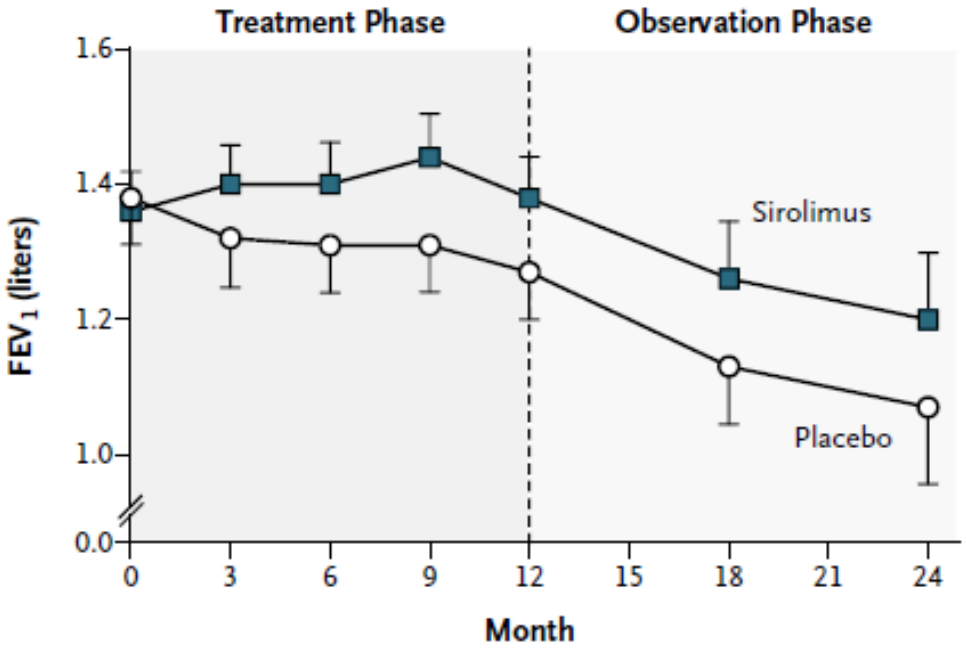
1. What are LAM and PLCH?

2. Are there useful biomarkers for diagnosis and follow-up?

3. Do we have a therapy?

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

The MILES trial: open issues

- What about patients with chylous effusions and lymphangioleiomyomas?

  - An observational study showed that sirolimus is effective on lung function and the size of chylous effusions and lymphangioleiomyomas

Taveira-Dasilva AM et al, Ann Intern Med. 2011

- What about long-term therapy?

  - An observational study showed sustained effects of Sirolimus on lung function and cystic lung lesions

Yao J et al, AJRCCM 2014

  - A Multicenter International Durability and Safety of Sirolimus (MIDAS) is ongoing

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

Definition of declining lung function: 90 mL/year?

- Problematic chylous effusions and lymphangioleiomiomas

McCormack FX et al, AJRCCM 2016

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

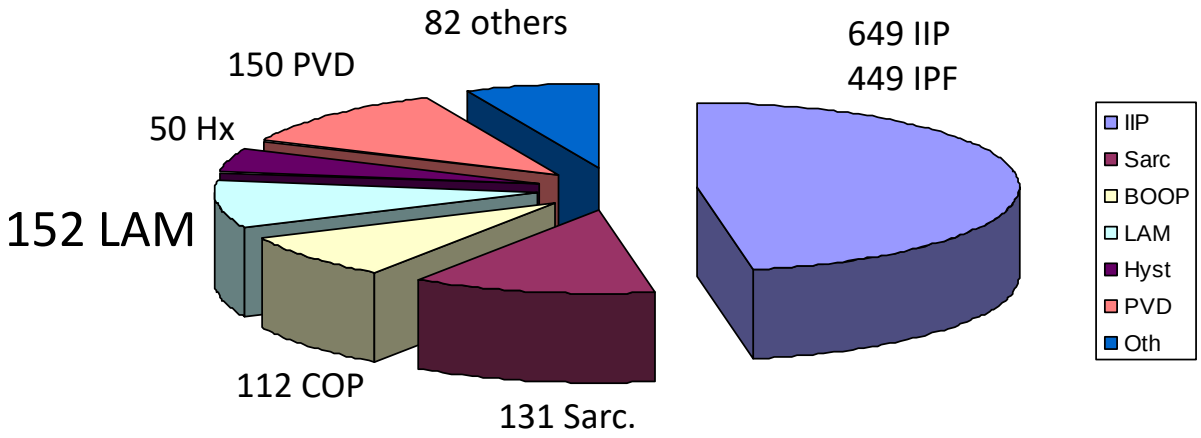
- Sirolimus is recommended
- Hormonal therapy is not recommended
- Doxycycline is not recommended

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

LAM

Ospedale San Giuseppe (1999- 2016)

Tot. 1326 pts



Mean age at diagnosis: 37 years
25 TSC-LAM

63 patients treated with Sirolimus

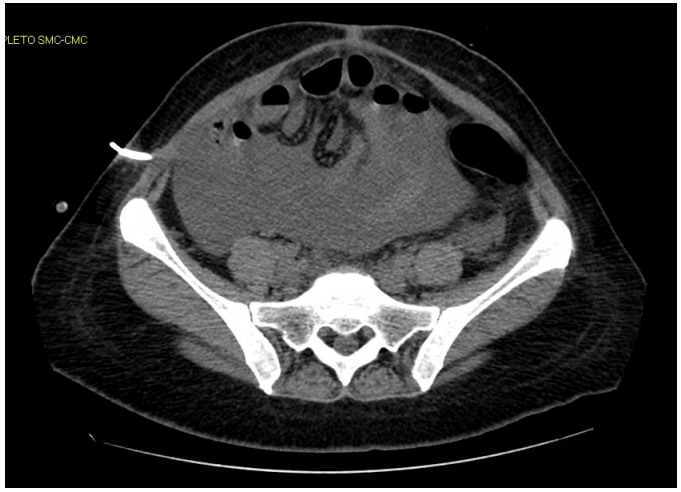
39 with > 1 year follow-up

- mean FEV1 change before treatment: - 100 ml/year
- mean FEV1 change during treatment: 20 ml/year
- 5 patients showed declining lung function after two year treatment period
- 1 patient died
- 2 patients discontinued the therapy because of adverse effects

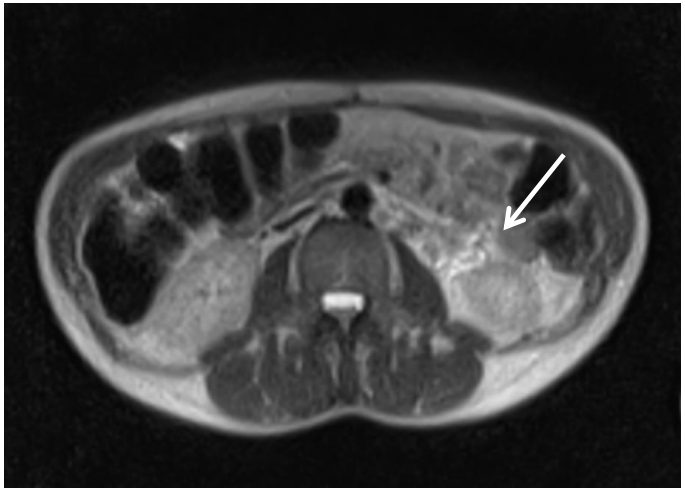
2 patients discontinued the therapy after few months because of adverse effects

Effectiveness of sirolimus in LAM

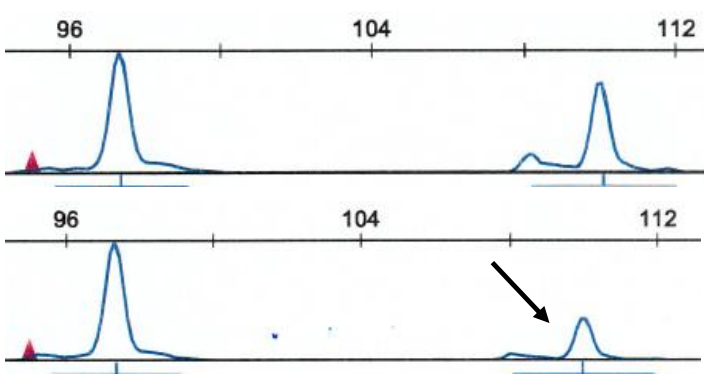
Before sirolimus

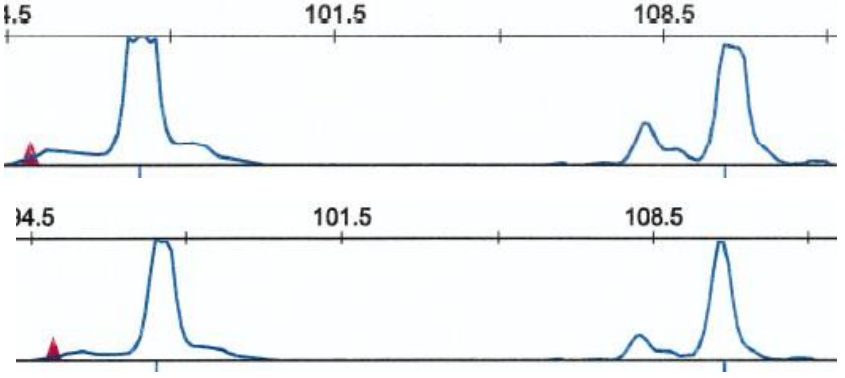


After 3 months of sirolimus



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

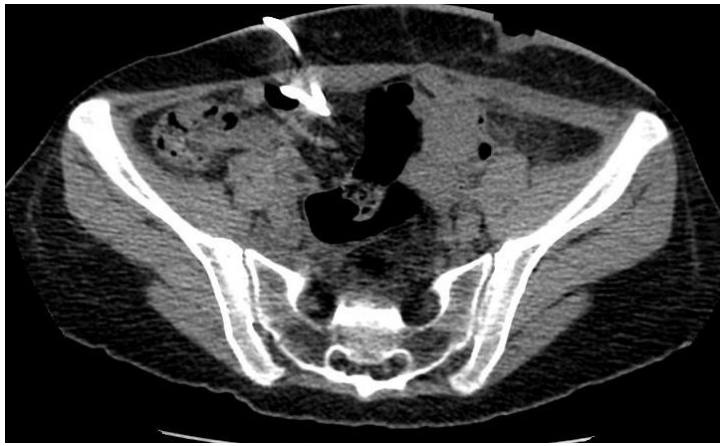




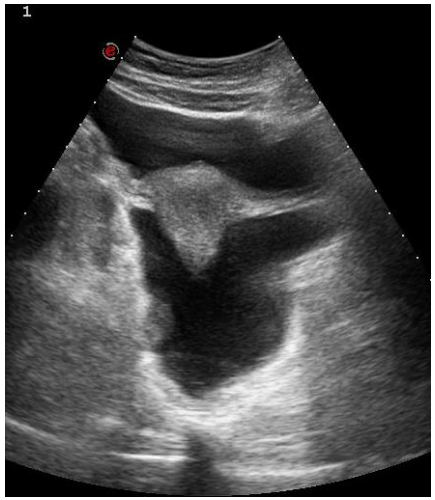
Harari S et al, Chest 2016

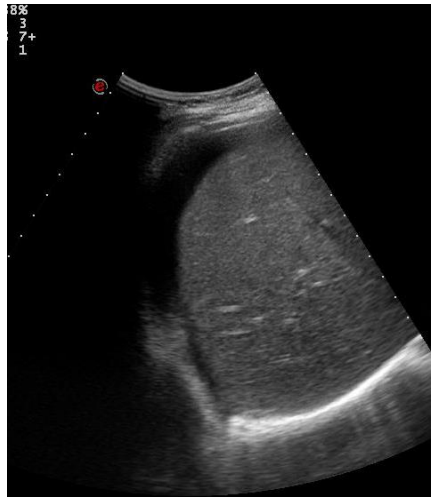
Ineffectiveness of sirolimus in LAM

Before sirolimus

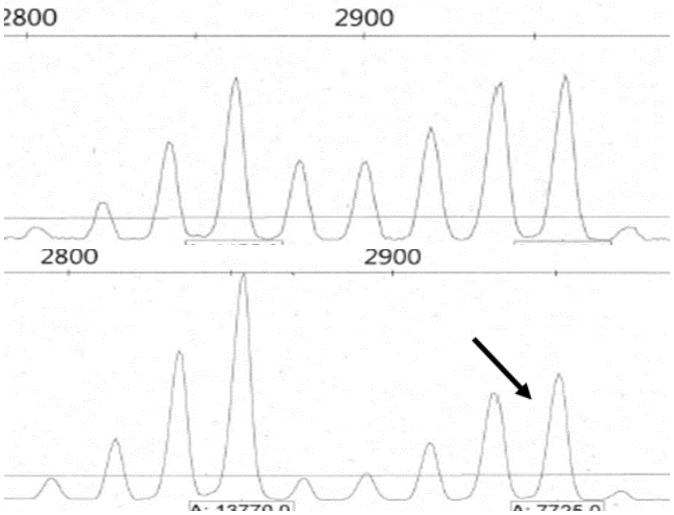


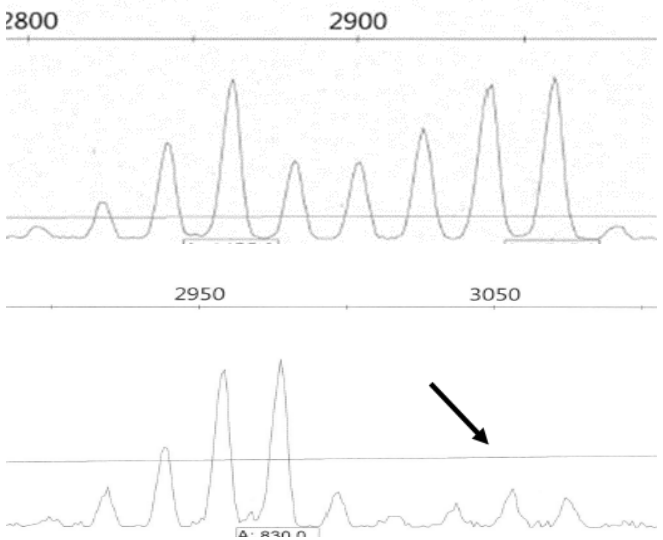
After 3 months of sirolimus



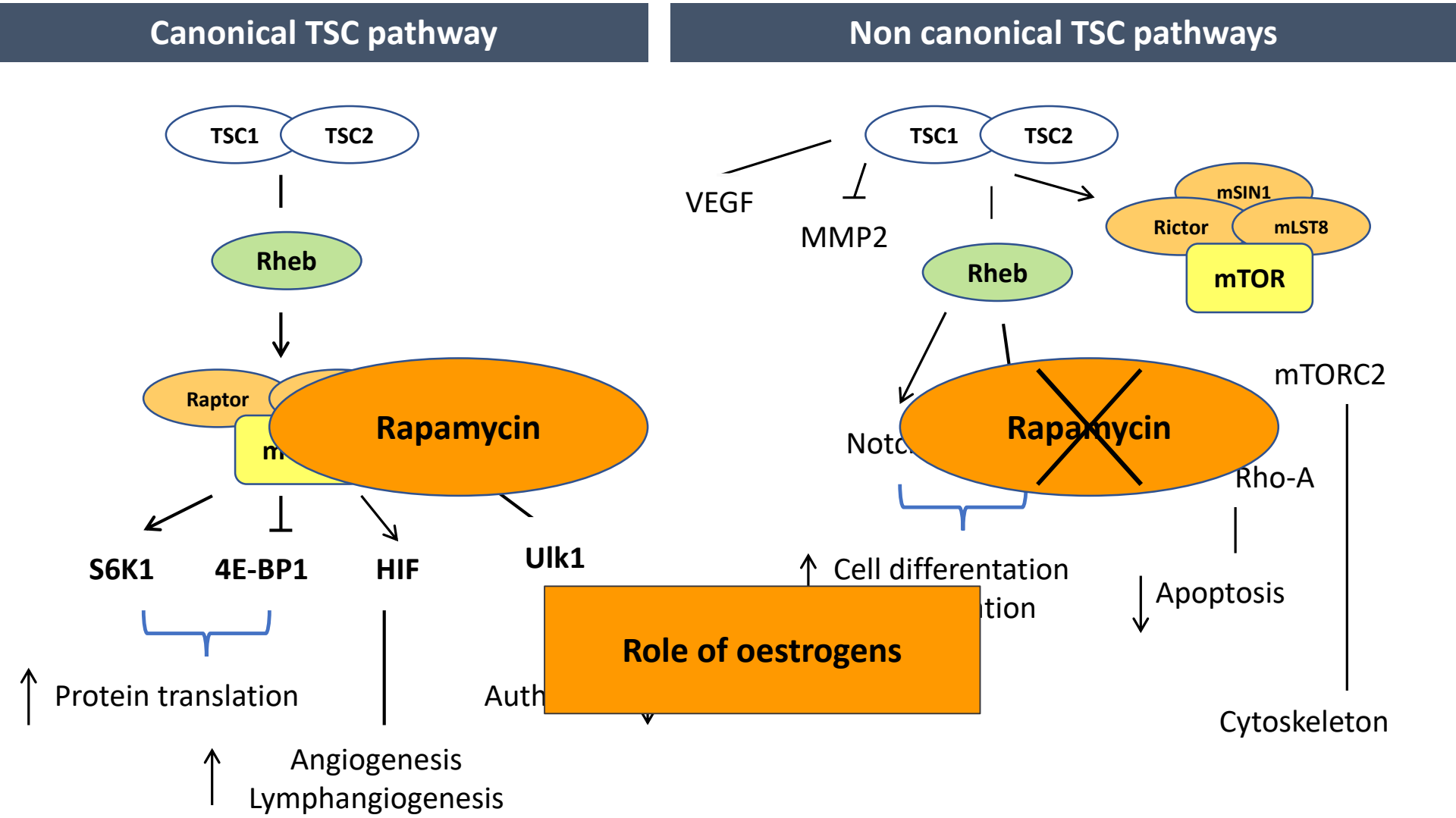


Serum VEGF-D: 776 pg/mL ⟶ 725 pg/mL





LAM - Pathogenesis



Treatment: the future

- Combination of mTOR and Autophagy inhibition

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

24 weeks treatment phase followed by 24 weeks observation phase
Most common adverse events: mucositis, headache, diarrhea
No drug-related SAEs

El-Chemaly S et al, Chest 2017

- Kinase inhibitors

A trial of Saracatinib (SLAM-2), an inhibitor of Src, is ongoing
Involved mechanism: proliferation, motility

- Combination of mTOR inhibition and statins

A retrospective study did not show that simvastatin enhances the beneficial effects of sirolimus therapy

Taveira da Silva et al, Chest 2015

A trial of mTOR inhibitors and simvastin is ongoing

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

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

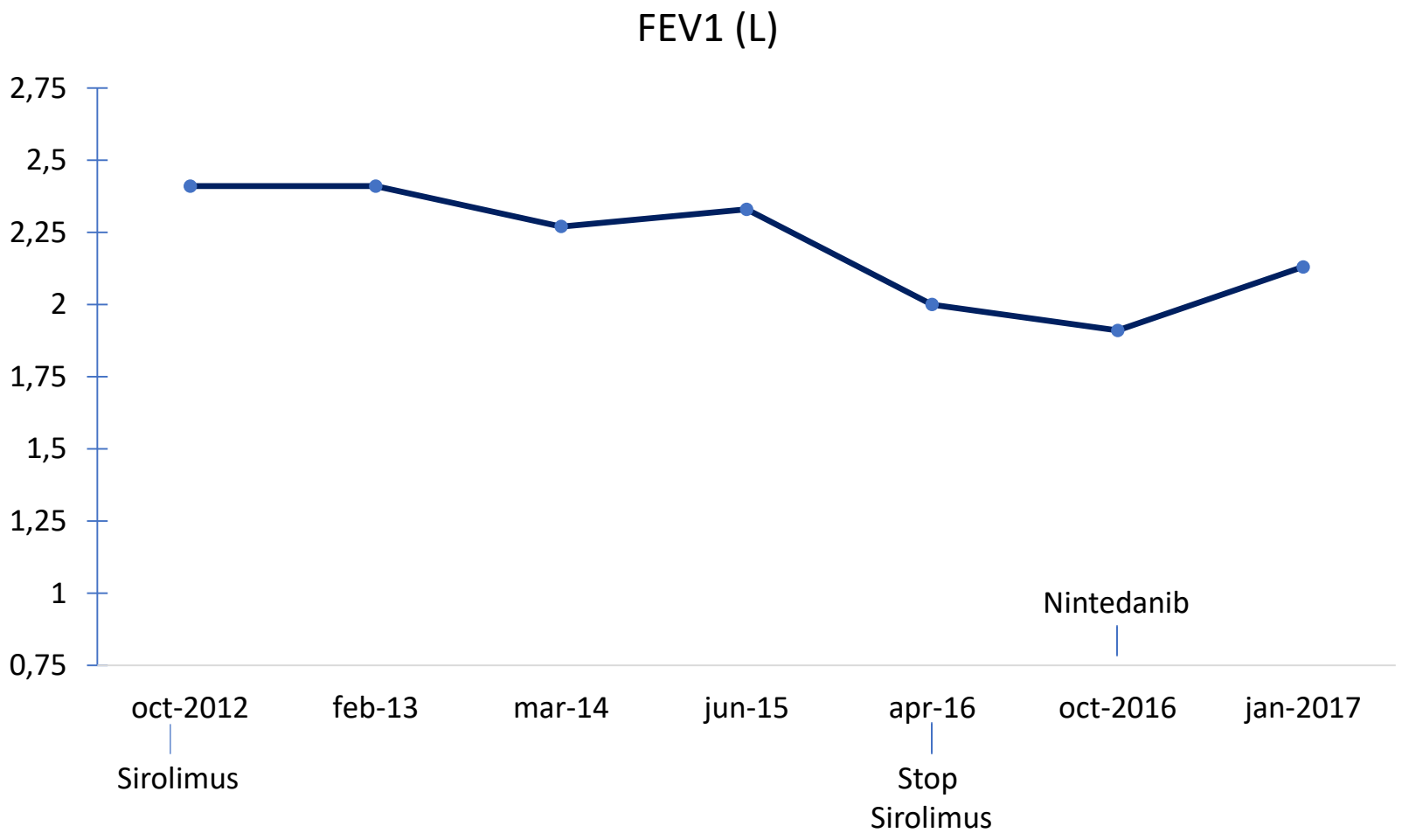
30 patients (10 patients enrolled already)
12 months treatment period
12 months follow-up

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



PLCH – Therapy

- Smoking cessation is mandatory!

In a recent prospective study persistence in smoking was associated with longitudinal decline in lung function, while smoking cessation for at least 6 months was associated with reduced lung function decline

Tazi A et al, Orphanet J Rare Dis 2015

- 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. It should only be considered in selected patients with pulmonary hypertension following right heart catheterization

Kiakouama L et al, ERJ 2010
Le Pavec J et al, Chest 2012

Cladribine in PLCH

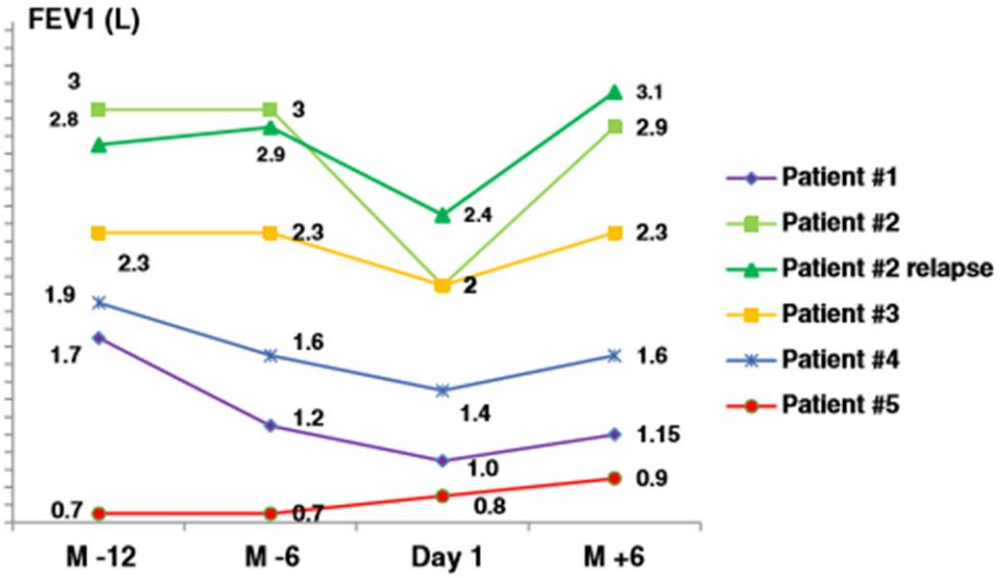
- Cladribine (2-chlorodeoxyadenosine) is a chemotherapeutic agent cytotoxic for lymphocyte and monocyte cells

- Case reports showed efficacy cladribine in PLCH

Effectiveness of cladribine therapy in patients with PLCH

A retrospective study of 5 patients with progressive, symptomatic PLCH with lung function impairment

FEV1 increased in all cases after therapy



Grobost et al, Orphanet Journal of Rare Diseases 2014

Cladribine in PLCH

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)

Inhibitors of Braf in PLCH

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Vemurafenib in Multiple Nonmelanoma Cancers with *BRAF* V600 Mutations

122 pts with $BRAF^{V600E}$ mutation–positive non-melanoma cancer

18 pts with ECD/LCH, 14 pts could be evaluated for a response:
Treatment with a BRAF inhibitor resulted in
- Complete response in 1 pt, partial response in 5 pts (RESPONSE RATE 43%)
- Stabilization of disease in 8 pts

None experienced disease progression during treatment

Hyman DM et al, N Engl J Med 2015

Conclusions

1.

To date LAM and PLCH are considered neoplastic diseases

2.

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

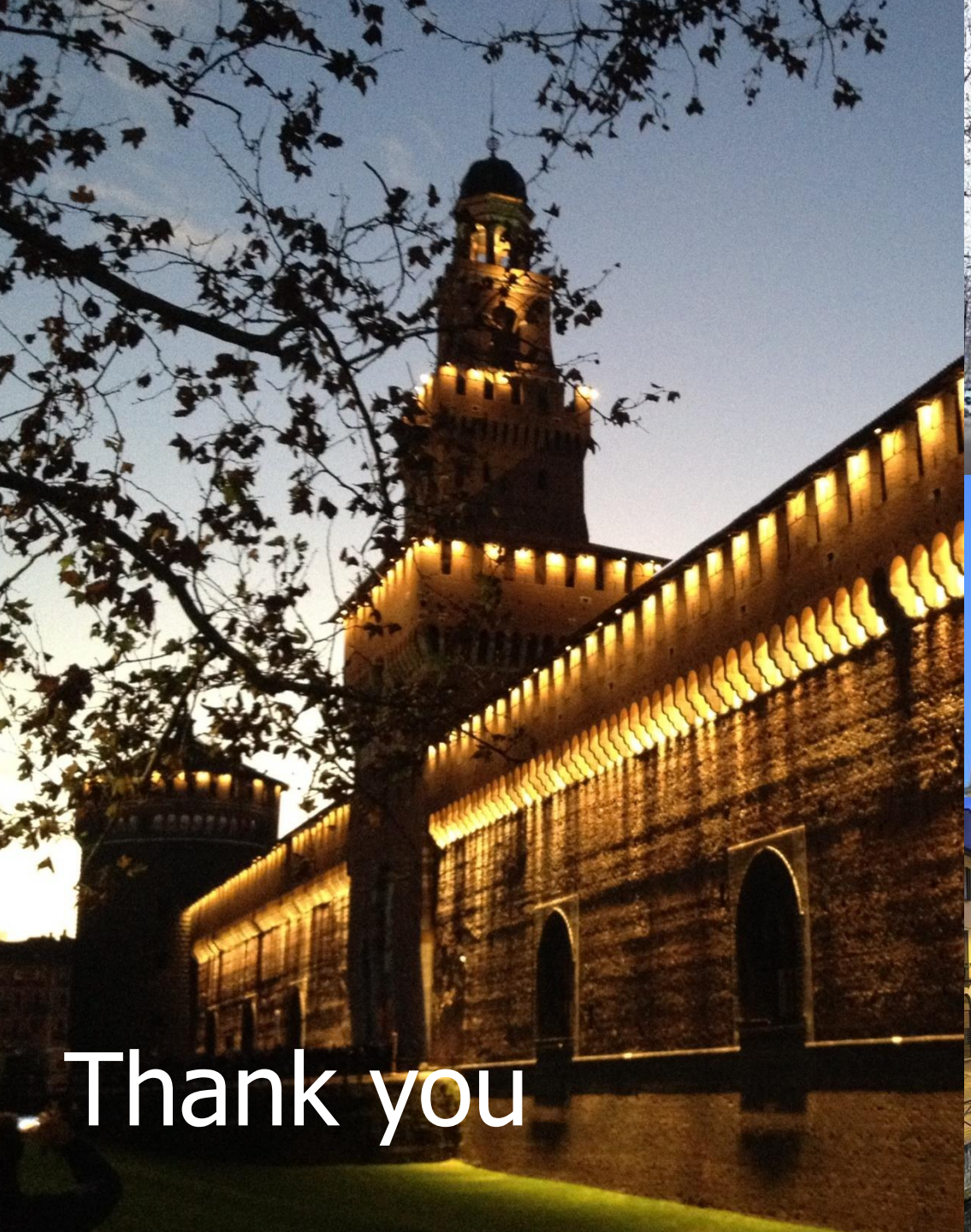
3.

An individualized combined treatment is likely the future for LAM patients

Cladribine and Braf inhibitors are the most promising treatments for aggressive PLCH

“What we know is a drop,
what we don't know is an ocean.”

Isaac Newton



Thank you