CORSO PNEUMOPATIE INFILTRATIVE DIFFUSE

AIPO ASSOCIAZIONE ITALIANA PNEUMOLOGI OSPEDALIERI

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Ospedale G.B. Morgagni - L. Pierantoni | Sala Pieratelli

BACKGROUND GENETICO NELLA LAM E NELLE ISTIOCITOSI POLMONARI

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Classification of Diffuse Cystic Lung Diseases

Gupta N et al, AJRCCM 2015

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.	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*	**Pulmonary Langerhans cell histiocytosis** 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

Lymphangioleiomyomatosis

- Lymphangioleiomyomatosis (LAM) is a multisystem disease affecting almost exclusively women, characterized by the formation of lung cysts, fluid-filled cystic structures in the axial and angiomyolipomas.

- LAM can be sporadic or occur in women with tuberous sclerosis complex (TSC)

- LAM results from the proliferation of LAM cells expressing smooth muscle-specific proteins (such as α-actin, vimentin, and desmin), and markers of melanoma cells (gp100 and MelanA/Mart1)

LAM - Pathogenesis

LAM is characterized by 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

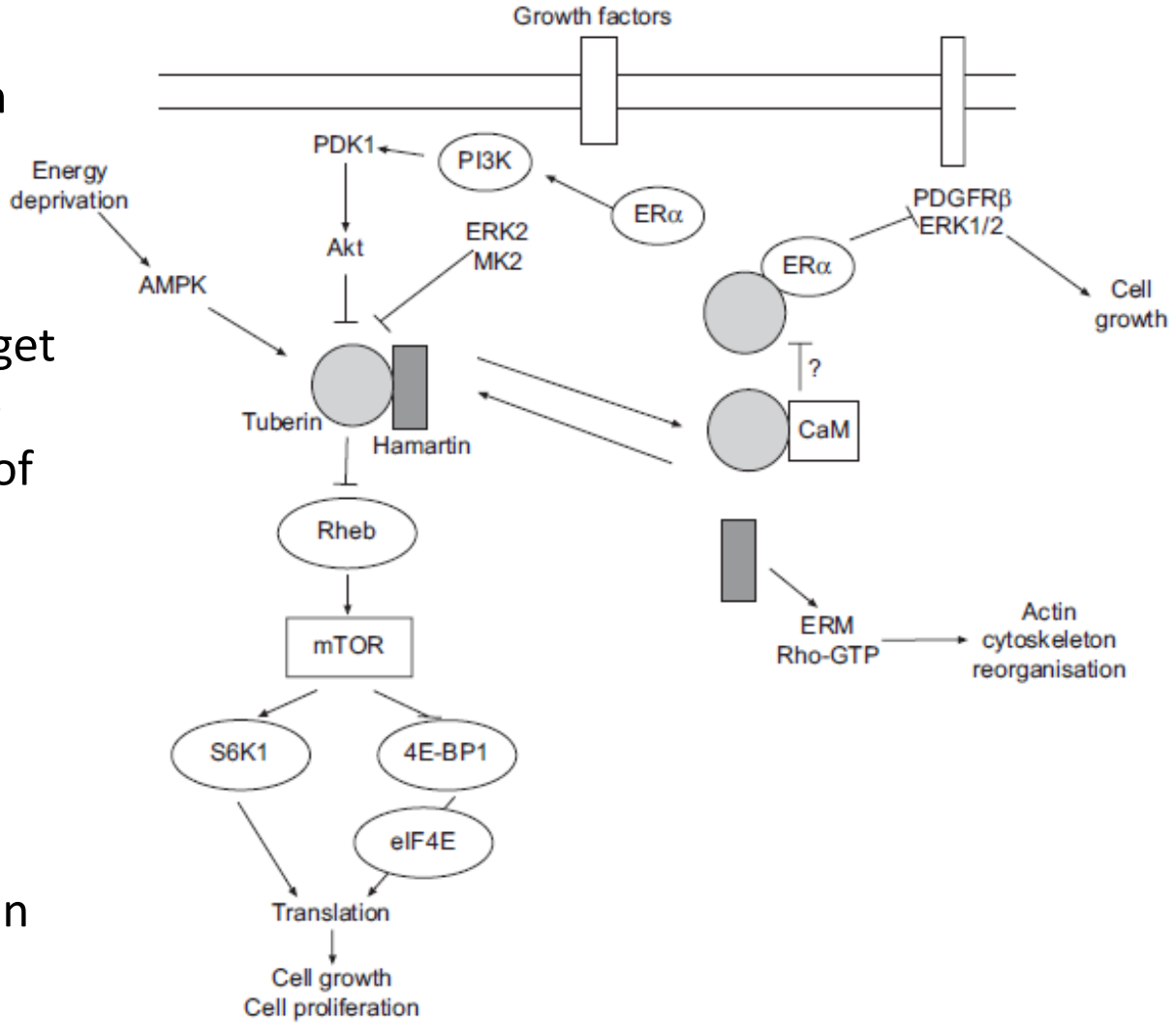
Tuberin is involved in the cell cycle and in cell growth and proliferation

Hamartin is thought to have a role in the reorganization of the actin cytoskeleton

LAM - Pathogenesis

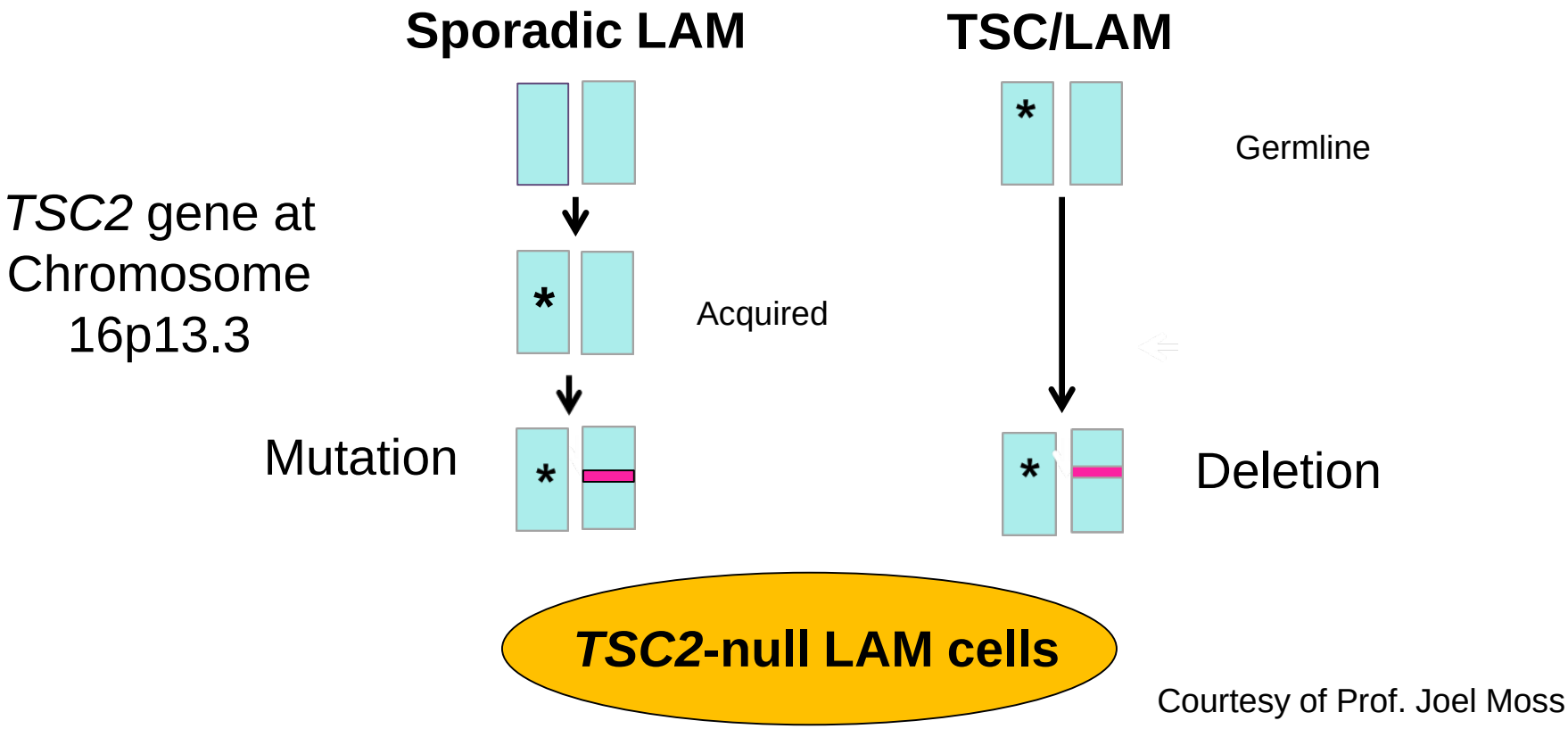
In vivo, hamartin and tuberin are part of a heterodimer acting upstream of the intracellular kinase mammalian/mechanistic target of rapamycin (mTOR), whose major function is regulation of cell growth and proliferation mediated by growth factors, energy and stress signals

Inactivating mutations of hamartin and tuberin result in a constitutive activation of mTOR pathway



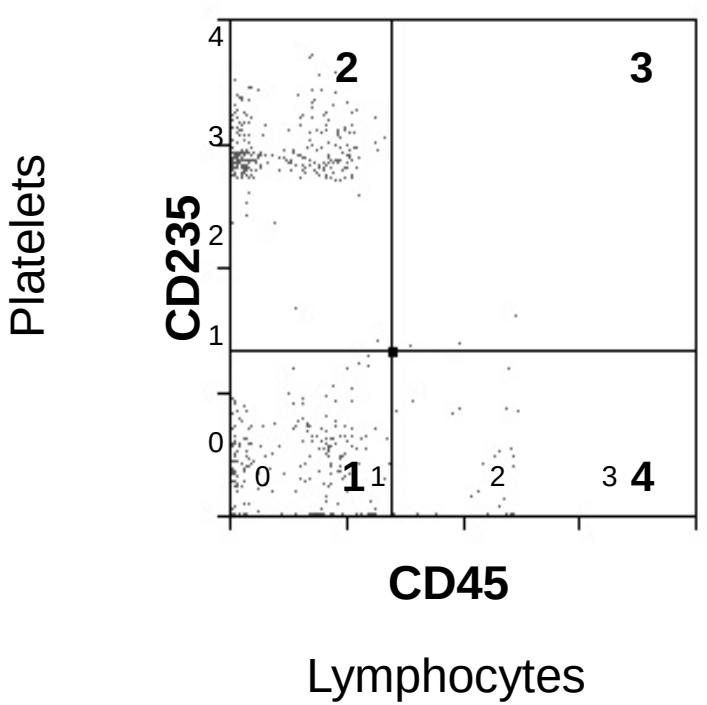
TSC2 Loss of Heterozygosity (LOH) and Knudson's Two-hit Model of Oncogenesis

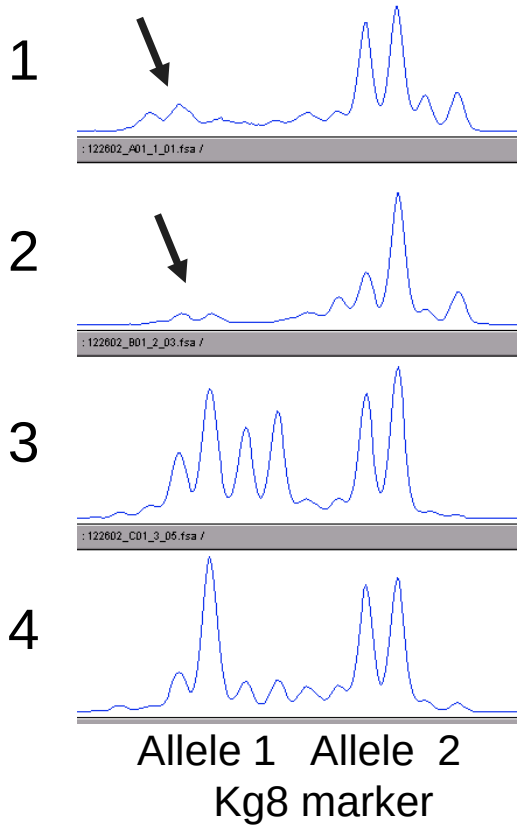
The current accepted model for LAM is consistent with Knudson's "two hit" hypothesis of tumour development: an initial mutation is followed by a second hit represented by loss of heterozygosity, causing the loss of function of gene products



Courtesy of Prof. Joel Moss

FACS and Chromosomal Analysis of Cells Isolated by Density Gradient Centrifugation





Courtesy of Prof. Joel Moss

TSC LOH

- 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.

Steagall WK et al, AJRCCM 2016

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

Zhang L et al, AJRCCM 2015

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	▲	□
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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

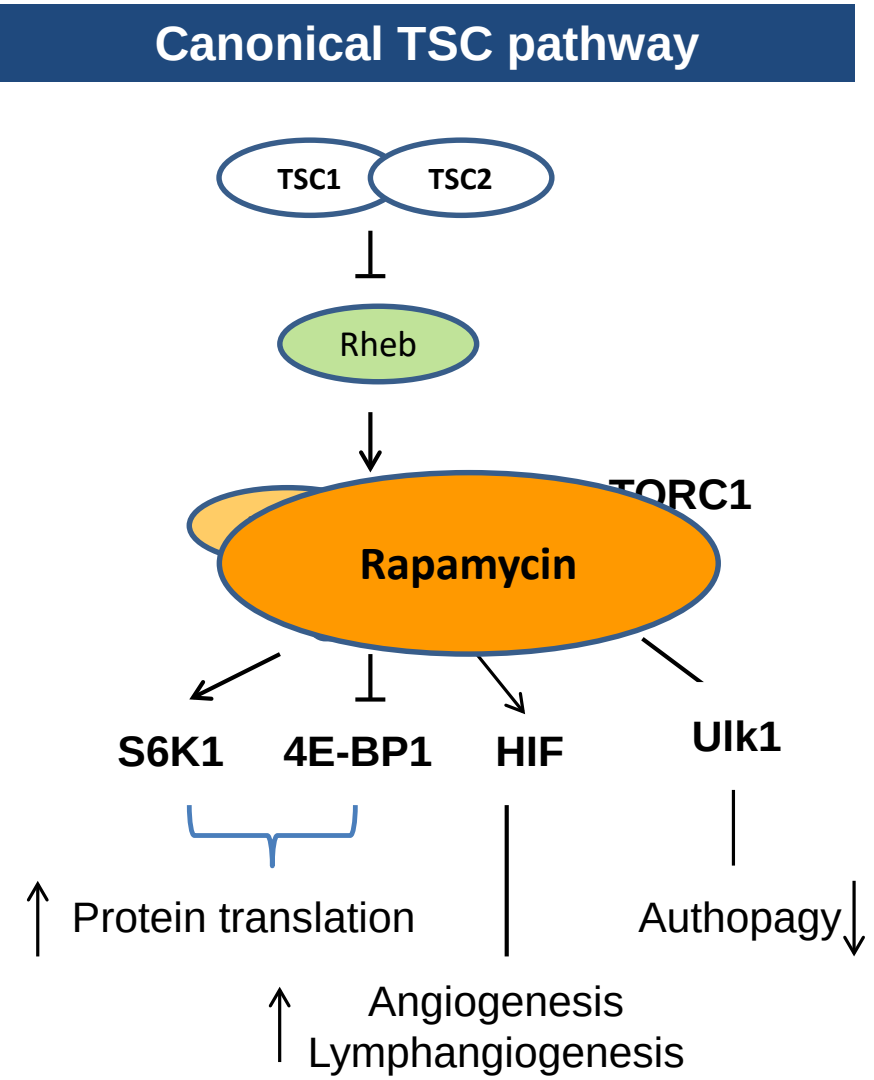
Blood (CD45-/CD235-, CD45-/CD235+): 91% (48/53)

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

Ongoing

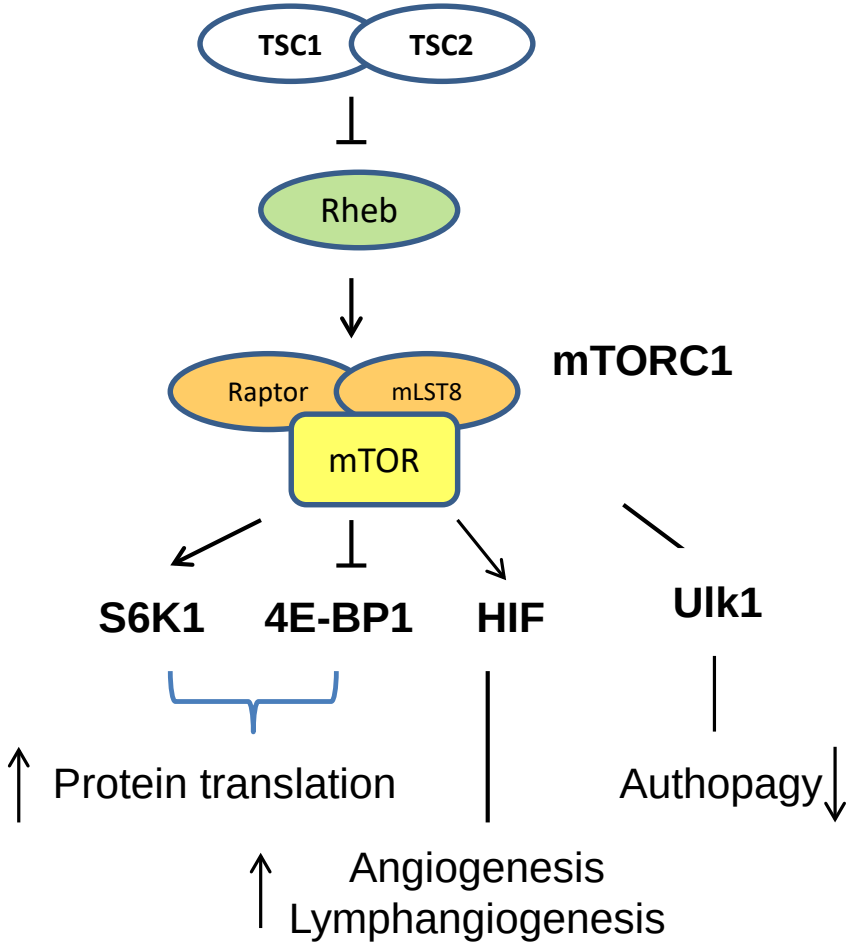
Search for TSC LOH in different cystic lung diseases to evaluate its possible role as a diagnostic biomarker

LAM - Pathogenesis

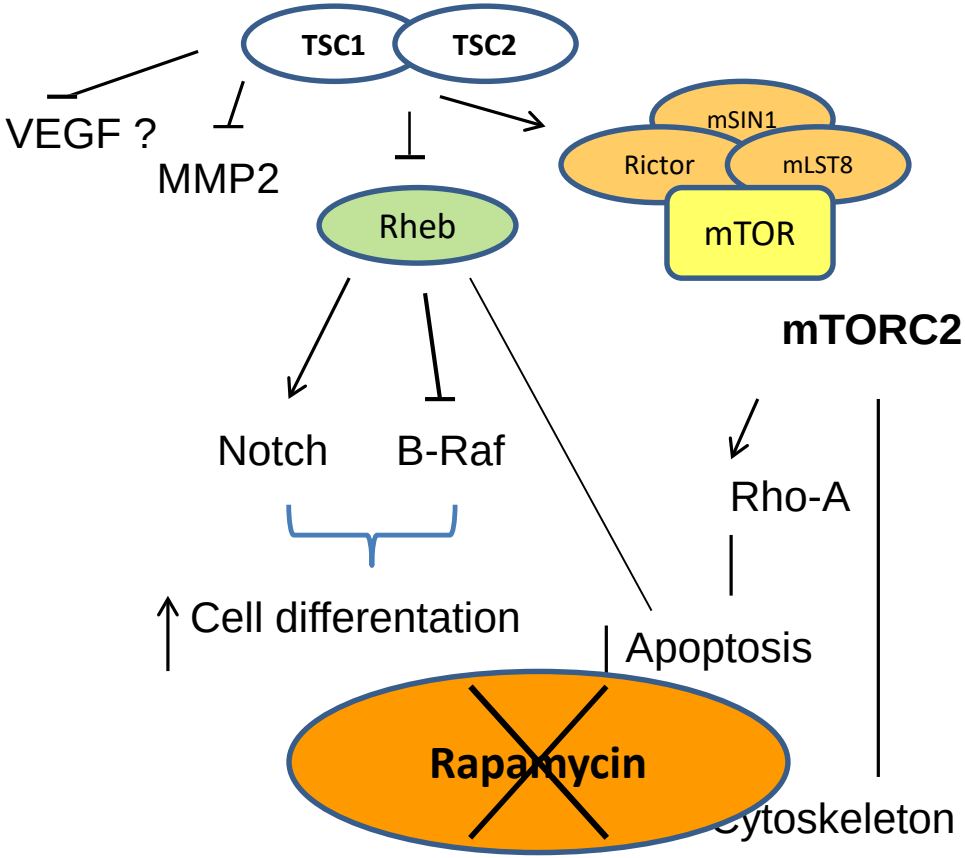


LAM - Pathogenesis

Canonical TSC pathway

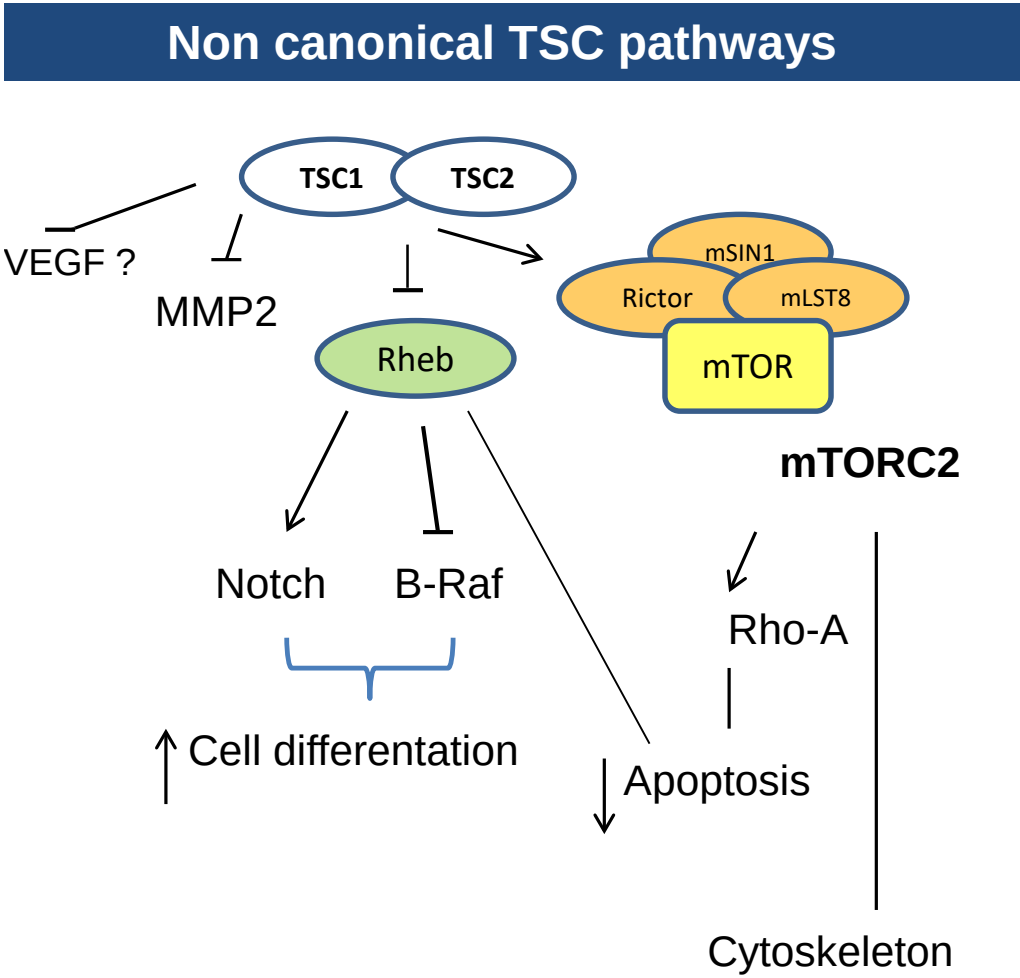


Non canonical TSC pathways

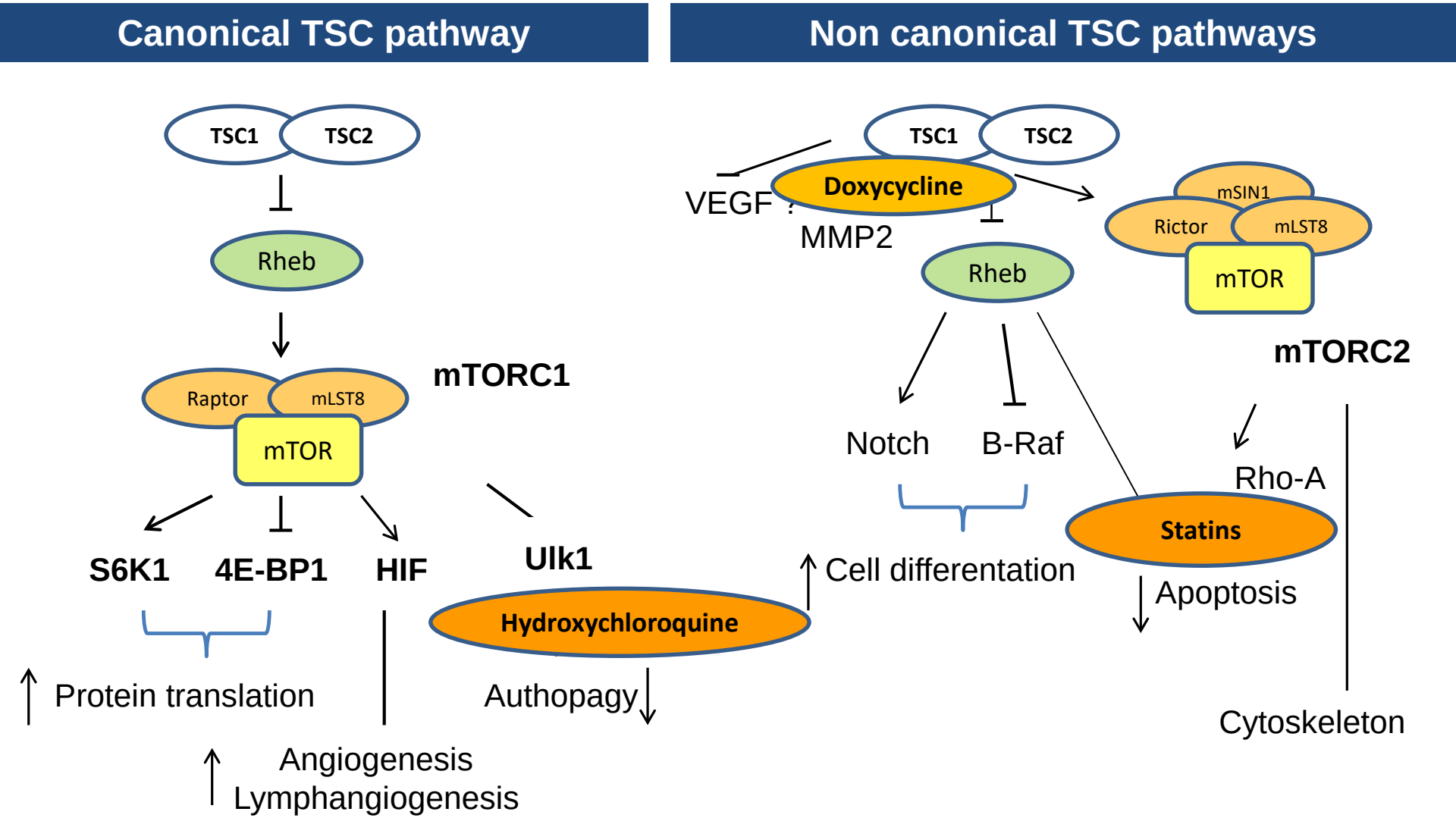


Possible mTORC1-independent mechanisms

- Regulation of the cytoskeleton via TORC2
- Regulation of the cytoskeleton and RhoA via hamartin
- Regulation of cellular differentiation and proliferation via B-Raf and Notch
- Rheb-independent regulation of MMPs by the hamartin–tuberin complex
- A TORC1-independent upregulation of VEGF?



LAM - Pathogenesis



serum VEGF-D in LAM

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

Source of VEGF-D

- Silencing TSC2 in human lung fibroblasts does not lead to increases VEGF-D levels:

  LAM cells may not be the direct source of excessive VEGF-D in LAM

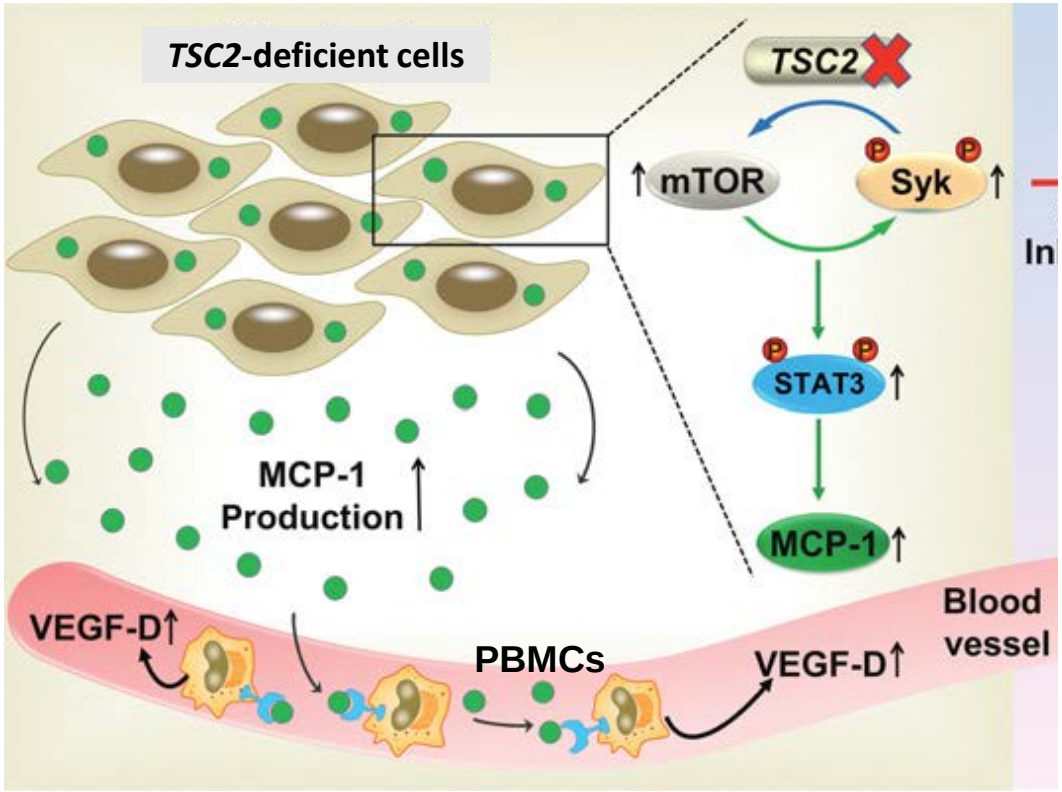
Cui Y et al, Mol Med 2014

- Syk (spleen tyrosine kinase) expression and activation in TSC2-deficient cells and in LAM lung lesions is deregulated

- Syk-dependent signals between TSC2-deficient cells and peripheral blood mononuclear cells (PBMC) induce VEGF-D expression

Cui Y et al, Cancer Research 2017

Source of VEGF-D



In TSC2-deficient cells activation of mTORC1 and Syk signaling induces MCP-1 overproduction via a STAT3-dependent manner. Subsequently, MCP-1 recruits and activates PBMCs, leading to increased VEGF-D expression

Cui Y et al, Cancer Research 2017

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, mesenchymal tumours composed of histologically and immunohistochemically distinctive perivascular epithelioid cells which present the coexpression of myogenic and melanocytic markers

<u>But the source of LAM cells is still unknown</u>

(Uterus? Kidneys? Lymphatics?)

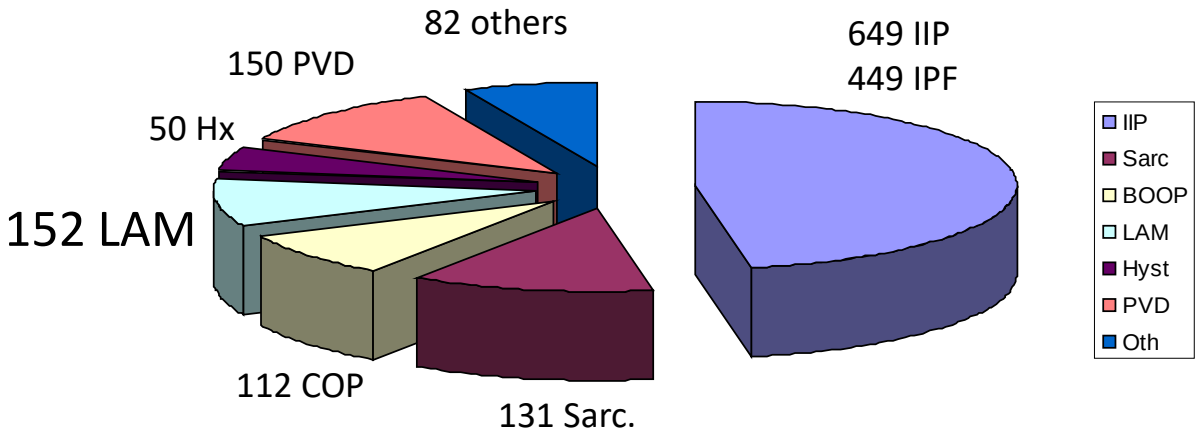
<u>LAM cells show no atypia</u>

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

LAM

Ospedale San Giuseppe (1999- 2016)

Tot. 1326 pts



Mean age at diagnosis: 37 years
25 TSC-LAM

63 patients treated with Sirolimus

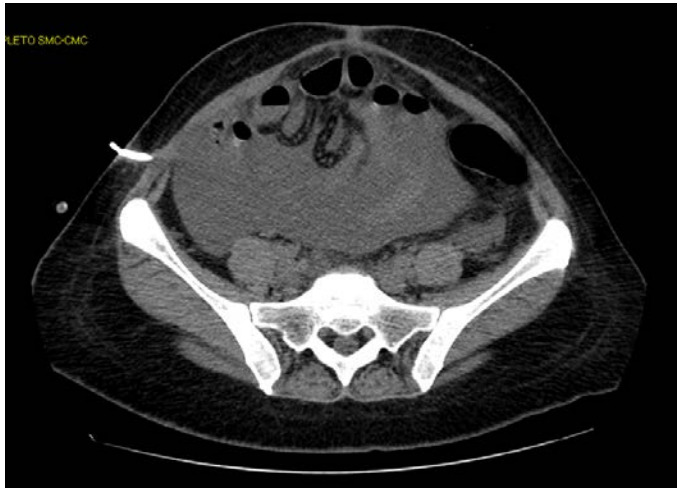
39 with > 1 year follow-up

- 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

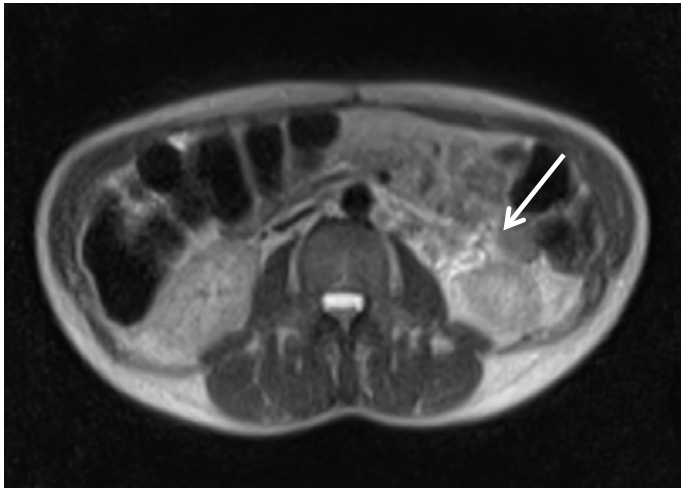
2 patients discontinued the therapy after few months because of adverse effects

Effectiveness of sirolimus in LAM

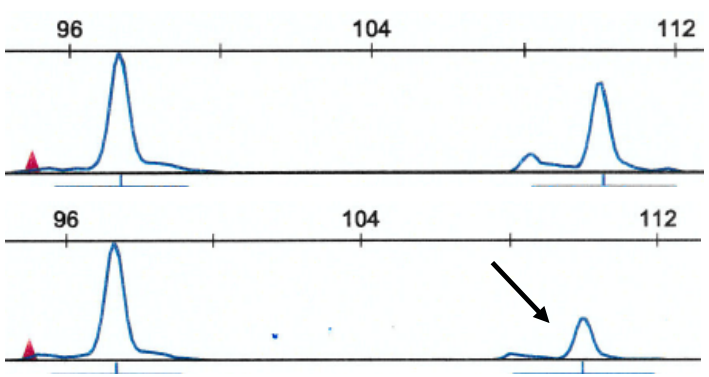
Before sirolimus

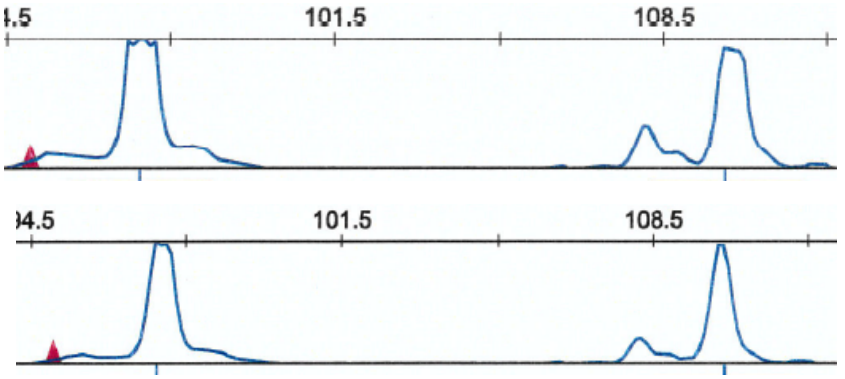


After 3 months of sirolimus



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

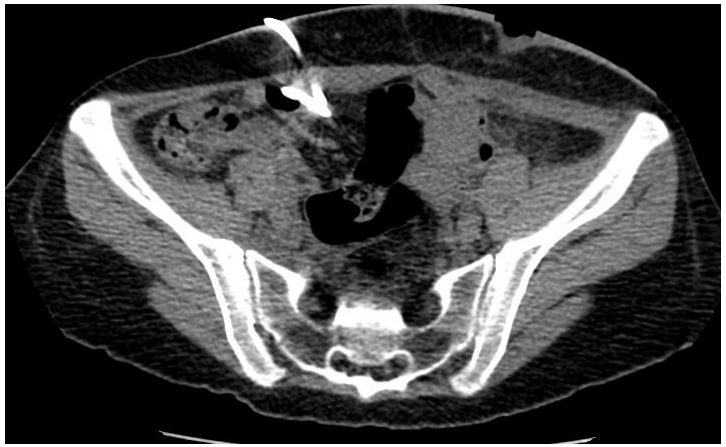




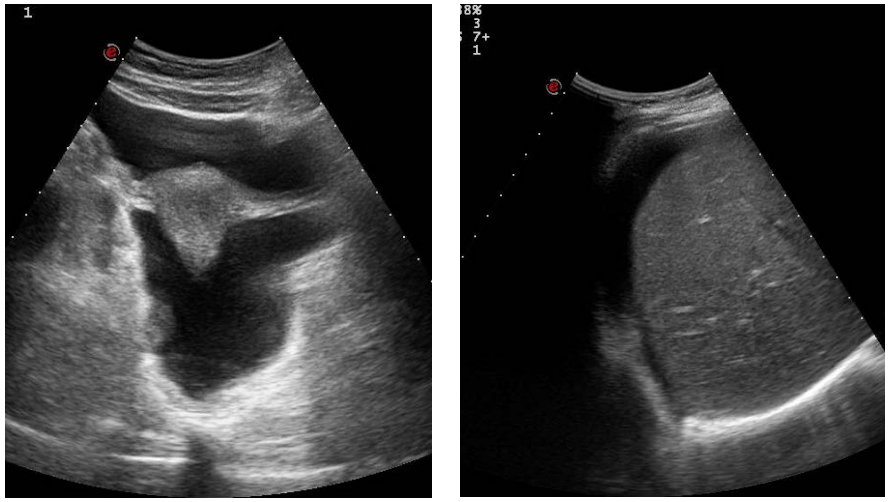
Harari S et al, Chest 2016

Ineffectiveness of sirolimus in LAM

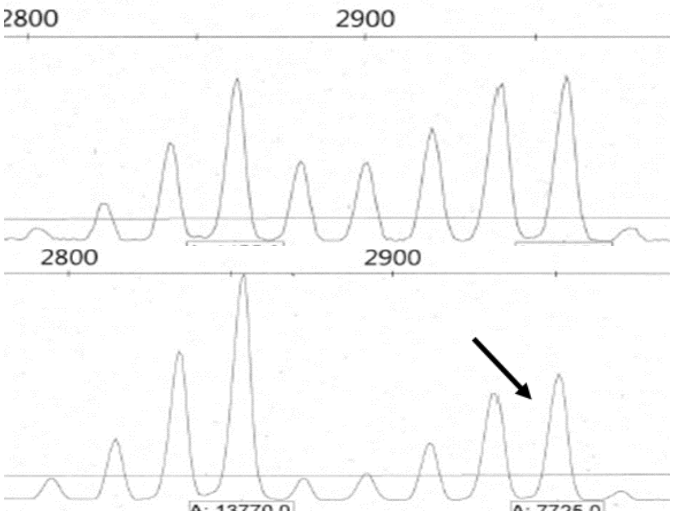
Before sirolimus

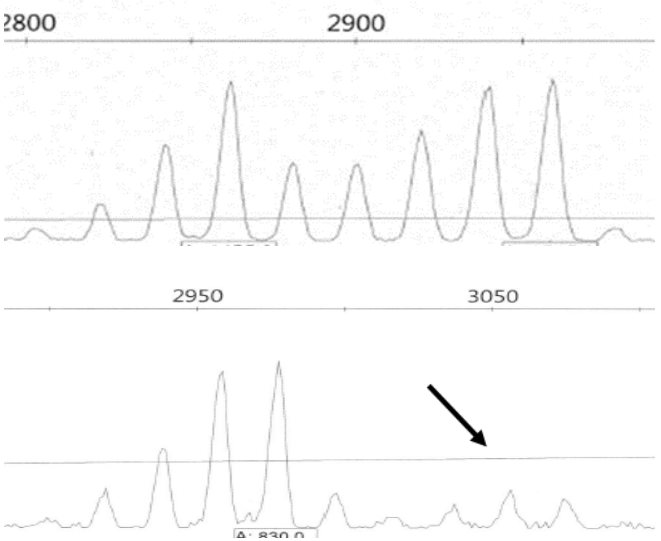


After 3 months of sirolimus



Serum VEGF-D: 776 pg/mL ⟶ 725 pg/mL





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

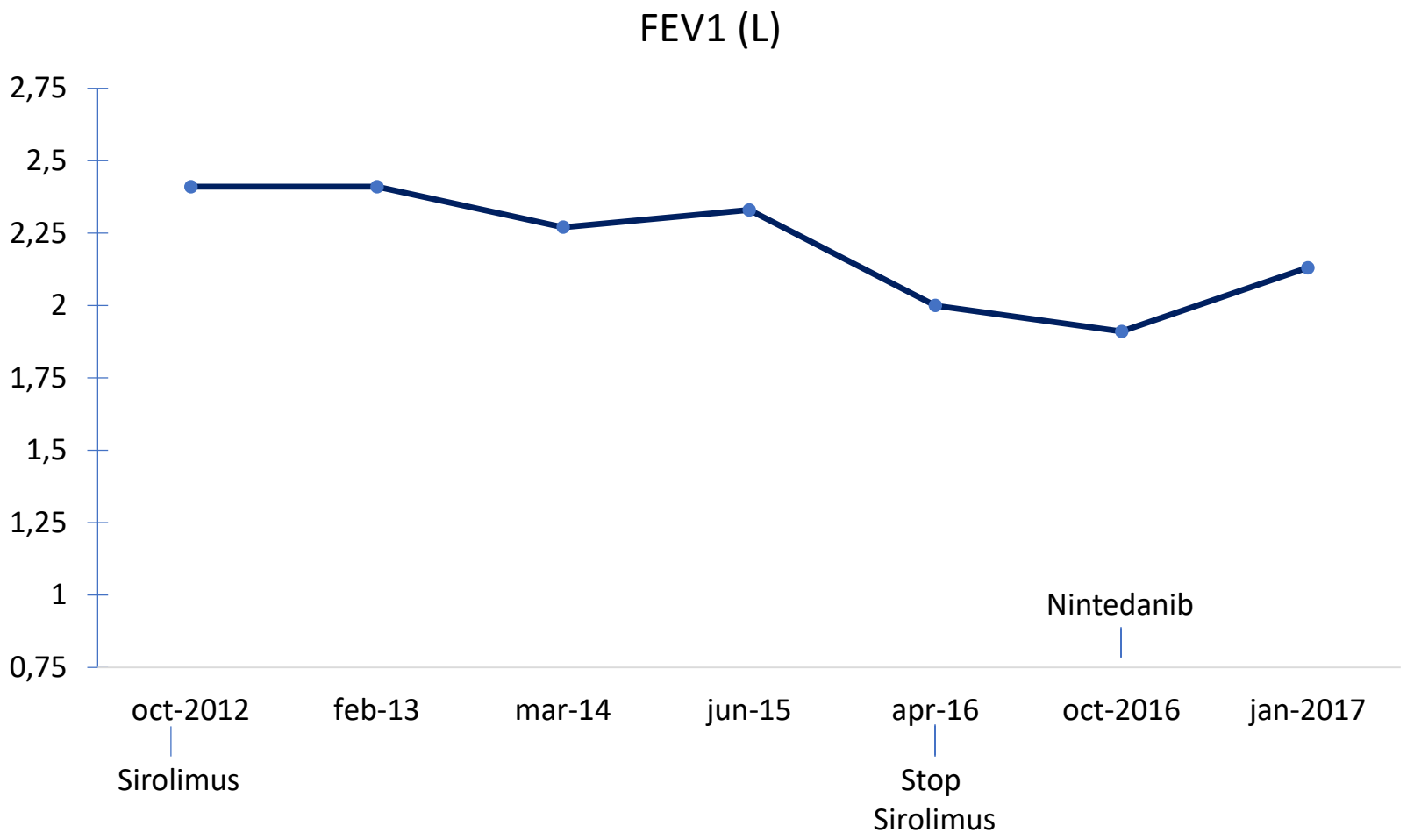
30 patients (10 patients enrolled already)
12 months treatment period
12 months follow-up

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



To summarize

1. LAM is characterized by inactivating mutations in TSC1/TSC2 genes

2. These mutations result in a constitutive activation of mTORC1 pathway, which is the main pathway involved in pathogenesis of the disease

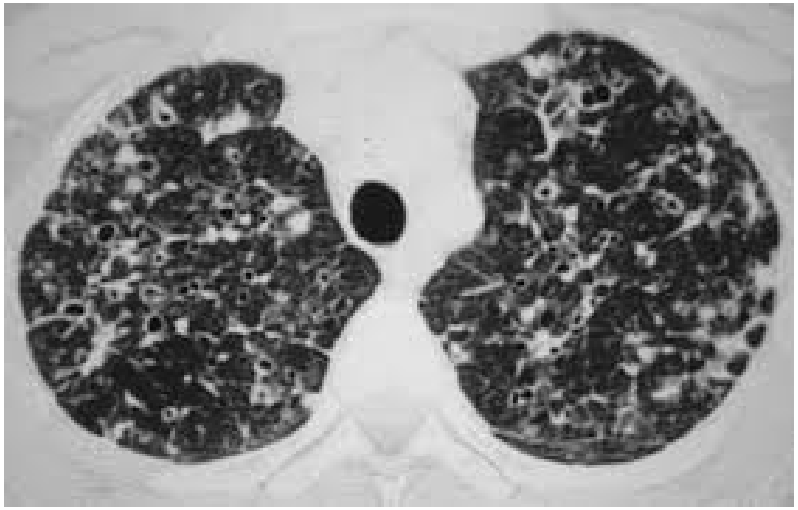
3. Other less known pathways are involved, thus suggesting the possible role of novel treatments

LANGHERANS CELL HISTIOCYTOSIS

It is a rare histiocytic disorder of unknown origin, affecting patients of different ages, but it is most common in children from 1 to 3 years old.

Lung may be involved as a single organ, typically in young smoker adults with equal gender distribution (Pulmonary Langerhans cell histiocytosis, PLCH) or, less frequently, in the systemic form of LCH .

The types of lung lesions vary with disease duration. In early disease, nodules and cavitated nodules are more numerous than lung cysts, while more advanced disease is often cystic in appearance.



CLINICAL FORMS OF LCH

- Localized: affecting one organ (often bone, skin and lung) characterized by a good prognosis with an occasional spontaneous resolution.

- Systemic: involvment of more than one organ or tissue (bone, skin, hypothalamic-pituitary system, lymph nodes, lungs, and more rarely central nervous system).

- An involvement of the so-called risk organs, such as liver, spleen and haematopoietic system, is associte to a worse prognosis.

Emilie JF *et al*. *Blood* 2016, 127: 2673-2681.

KEY ELEMENTS ABOUT THE PATHOGENESIS of PLCH

1) the reactive vs clonal / neoplastic nature of the disease;

2) the mechanisms of accumulation of large numbers of CD1a+ cell in bronchiolocentric loosely-formed granulomas;

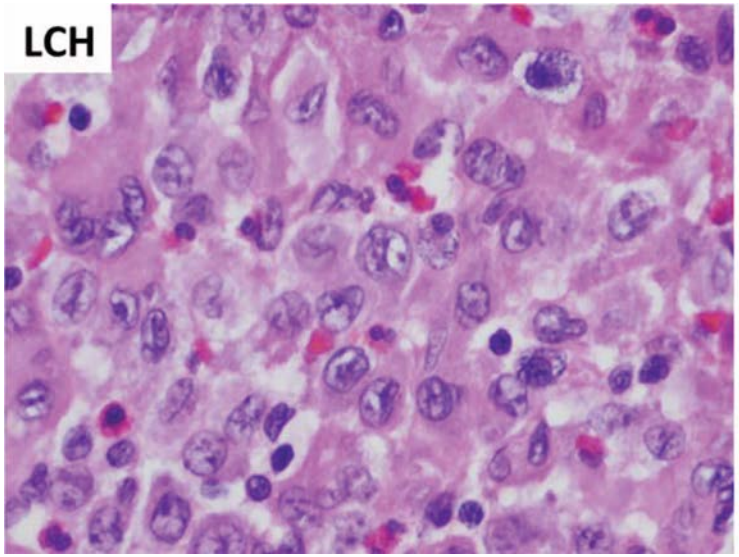
3) the capacity of these granulomatous lesions to destroy and remodel surrounding tissues;

4) the role of smoking in adult PLCH.

Is it a reactive disease?

This hypothesis is supported by:

- the finding of the presence of CD1a+/CD207+ histiocytes among an inflammatory background including variable numbers of lymphocytes, eosinophils, and macrophage.
- the absence of pathological features like mitotic figures and of recurrent cytogenetic abnormality in the CD1a+ cells of these lesions.
- the frequent spontaneous resolution after the smoking cessation.



Brabencova E *et al*. *Am J Pathol* 1998;152:1143-1149

Is it a neoplastic disease?

A clonal nature of LC has been found in cell derived from extrapulmonary lesions of focal or systemic forms of LCH.

Clonality in immune cells does not necessarily imply malignancy, and failure to identify genetic abnormalities in systematic analyses of LCH lesions cautioned against classification of LCH as a neoplastic or malignant disorder.

A major breakthrough supporting this hypothesis came with discovery of recurrent somatic proto-oncogen ***BRAF-V600E*** mutations in histiocytes of LCH lesions

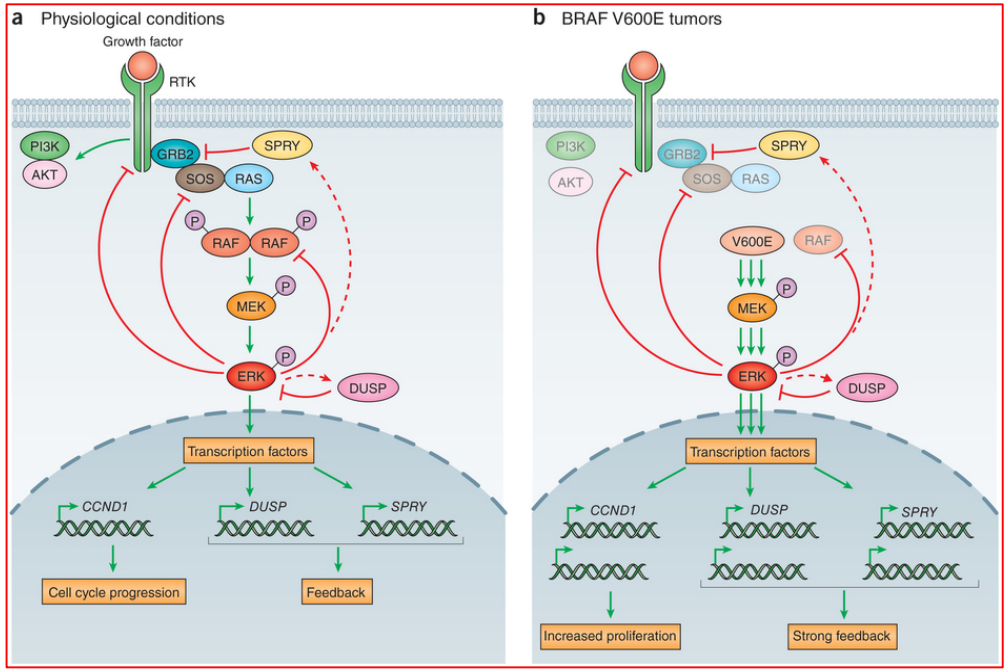
BRAF-V600E

BRAF is a central kinase of the RAS/RAF/MEK pathway, which essentially lead to the phosforilization of ERK, involved in numerous cell functions including cell proliferation and migration and is frequently mutated in various cancer cells

(Davies et al, 2002).

The BRAF V600E mutation results in a constitutive, RAS-independent activation of the downstream kinases extracellular signalregulated kinase (ERK) and mitogen-activated protein kinase (MAPK)/ERK kinase (MEK)(Maurer et al, 2011).

Both downstream kinases are highly activated in LCH cells with BRAF V600E mutation, supporting the potential functional relevance of the mutation in LCH (Badalian-Very et al, 2010; Chakraborty et al, 2014)



BRAF- V600E and LCH

BRAF-V600E mutation has been described in the sample of the lesions of:

- 38 to 69% of LCH patients (Badalian-Very G *Blood* 2010, Satoh *Pediatric Disease* 2012, Sahm *Blood* 2012, Hervier *Blood* 2014)
- 7 of 25 (28%) of patient with PLCH (Rodean *Am J Sur Pat* 2014)
- 54% to 82% of patients with Erdheim Chester Disease (Haroche *Blood* 2012, Hervier *Blood* 2014)

In children with severe systemic forms of LCH, the presence of the $BRAF^{V600E}$ mutation was not only present in tissue lesions (somatic mutation), but also in circulating and sometimes bone marrow precursors of dendritic cells infiltrating LCH granulomas

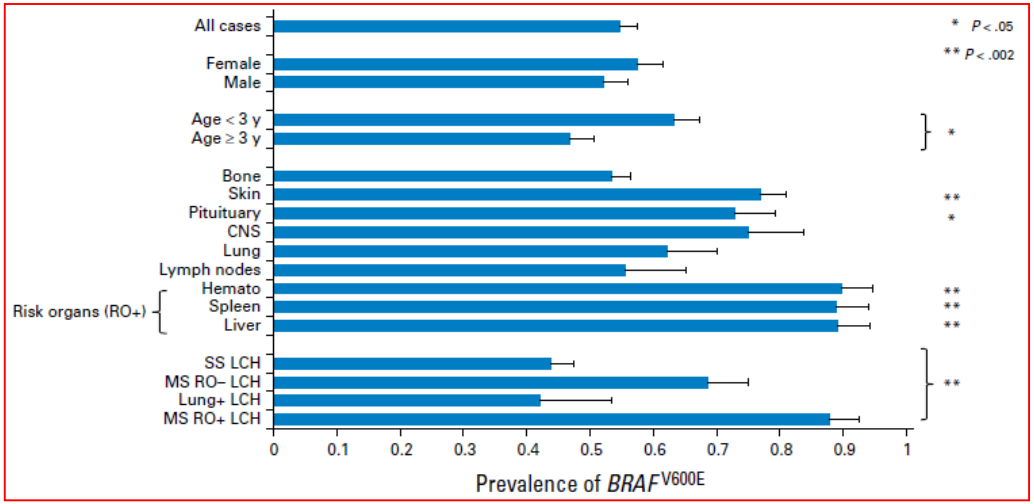
(Barres et al. *The Journal of experimental medicine* 2015)

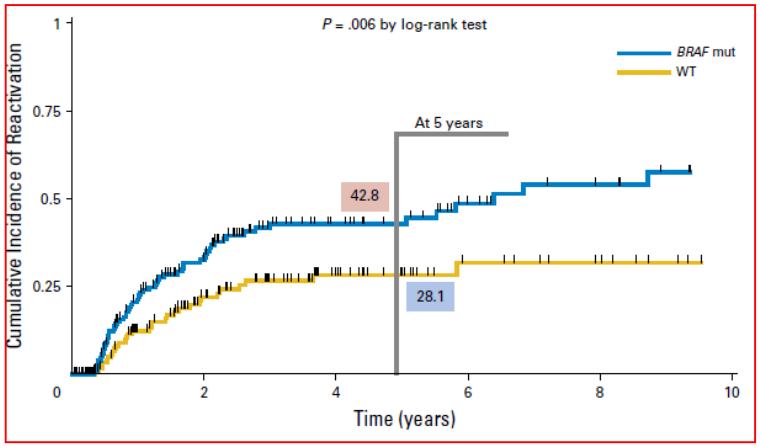
BRAF-V600E and clinical manifestation

BRAF-V600E expression in LCH and PLCH samples is related to a worse prognosis and a poor short-term response to first line treatment.

BRAF-V600Emutations was investigated in 315 pts in a French LCH cohort. The presence of the mutations in the 54.6% was related to a higher multisystemic involvement (included organ risk involvement).

Pts with the mutation showed more frequently resistence to combined vinblastine and steroids treatment and a higher 5-yrs reactivation rate.

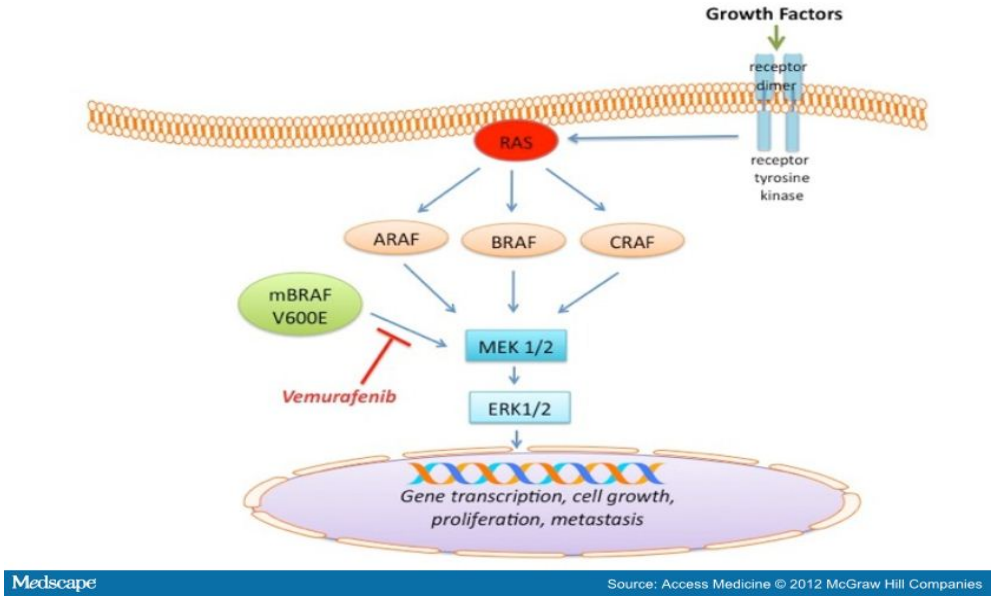




Hertier S. et al. JCO 2016

Therapeutic options in BRAF^{V600E+} patients: Vemurafenib

Vemurafenib is an inhibitor of mutant BRAF, and has some efficacy against both BRAF^{V600E} associated melanoma and hairy-cell leukemia



Vemurafenib and LCH

In 3 patients with multisystemic and refractory ECD with the $BRAF^{V600E}$ mutation the treatment with vemurafenib led to rapid clinical and biologic improvement just 1 month after treatment. (Haroche Blood 2013).

In 8 patients with multisystemic $BRAF^{V600E+}$-ECD, refractory to first-line treatment was observed an improvement of general symptoms and a persistent response to vemurafenib, with a median follow-up time of 10.5 months (range, 6 to 16 months) (Haroche JCO 2015).

In a patient with LCH and a CNS involvement an objective reduction of the lesions at the MRI was observed. Skin adverse effects were frequent and severe. (Haroche JCO 2014)

NOT ONLY BRAF-V600E

Besides the frequent BRAF V600E mutation, single case reports have described additional mutations/polymorphisms within the BRAF gene locus with potential functional consequences, including the somatic mutations BRAF V600D, BRAF 600DLAT and the germline mutation/poylmorphism BRAF T599A (Satoh et al, 2012; Kansal et al, 2013).

A complex compound somatic mutation in ARAF with enhanced kinase activity in vitro has also been described in a single patient (Nelson et al, 2014). However, extended studies are necessary to elucidate the frequency and significance of these additional RAF mutations in LCH.

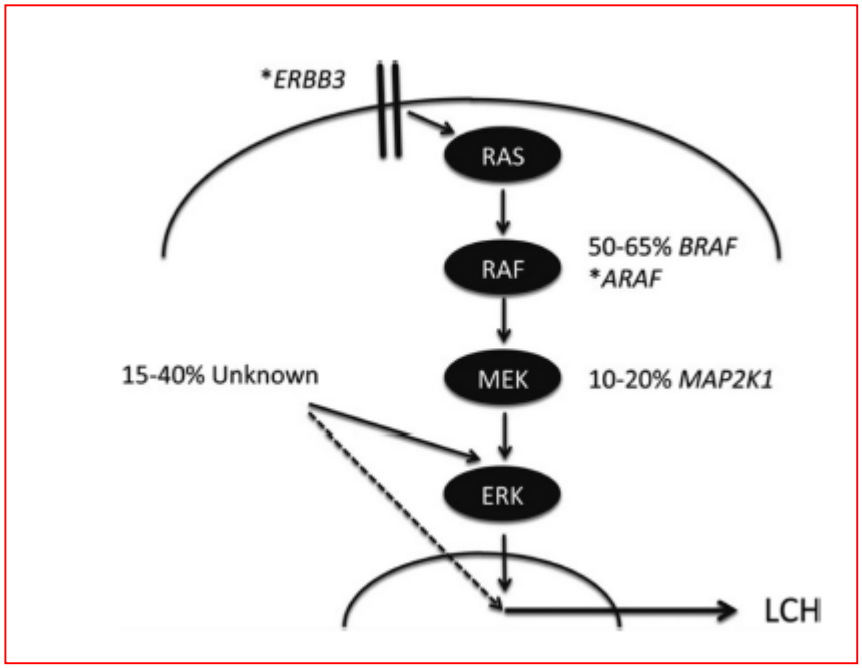
MAP2K1 is another recurrent mutated gene locus, also a member of RAS/RAF/ERK pathways, found in 33 to 50% cases of wt-BRAF cases

(Brown NA, Blood 2014, Nelson DS Genes Chrom Cancer 2015)

Interestingly, BRAF and MAP2K1 were mutually exclusive.

MAP2K1 mutations results in the constitutive phosphorylation of the downstream targets MAPK3 (also termed ERK1) and MAPK1 (also termed ERK2) in vitro comparable to the effects observed by the expression of BRAF V600E (Chakraborty et al, 2014)

Mutations of other genes that transcribe MAPK pathway proteins, specifically in ERBB3 and within the ARAF locus were observed (Chakraborty et al, 2014) though their frequency and functional significance remains to be proven in larger series



Role of smoking

Smoking interactions on gene mutations has not been investigated, so far, in PLCH

Children with extrapulmonary LCH who subsequently develop PLCH during adolescence or adulthood are often smokers (Bernstrand C. Acta Ped 2000)

Smoking induce Cd1a+ cell accumulation even in lungs of healthy smokers and stimulate locale production of different chemokines and promotes the survival of dentirtic cell via anti-apoptotic mechanism

Osteopontin seems to play a role in PLCH ait has been found in large quantities in BAL of PLCH pts comared to smoker controls. It actracts monocytes/macrophaces and dentritic cell and in rat models its overexpression induces lesions similar to those of PLCH

To summarize

1. RAS/RAF/ERK/MEK/ERK pathway play an important role in the pathogenesis of LCH with a multi- or single-organ involvement (included PLCH)

2. BRAFV600E has been largely found in different samples of LCH, ECD and PLCH. Its espresionis related to the severity of the disease, response of first line treatment and to increase of relapse after treatment

3. MAP2K1 and ARAF mutations are involved in the activation the RAS-RAF-MEK -ERK pathway in the setting of wild-type BRAF suggesting new target for goal therapy

4. Although smoke is involved in the pathogenesis of PLCH, interaction with genes has found, so far.