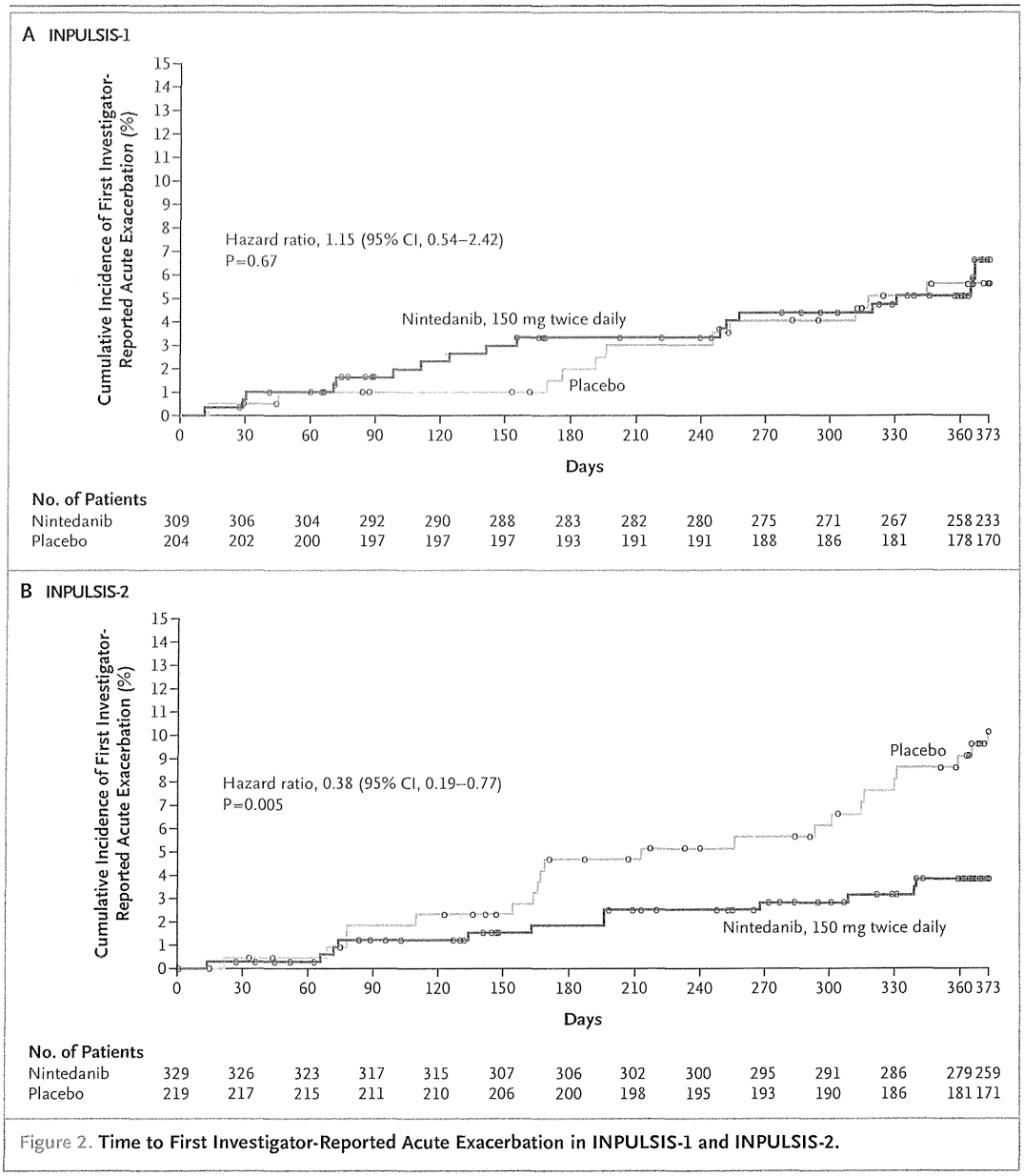

Figure 2. **Time to First Investigator-Reported Acute Exacerbation in INPULSIS-1 and INPULSIS-2.**

in INPULSIS-1; in INPULSIS-2, the difference between the groups was not significant.

A prespecified pooled analysis of the primary end point showed a significant treatment effect, (between-group difference in the annual rate of FVC change, –109.9 ml [95% CI, 75.9 to –144.0]) (Fig. S3A in the Supplementary Appendix). Pooled data on the absolute change from baseline in FVC are shown in Table S5 and Figure S3B in the Supplementary Appendix. A prespecified pooled analysis of data from the two trials showed that a significantly greater proportion of patients in the nintedanib group than in the placebo group had an FVC response with both definitions of a response (a decline in the percentage of predicted FVC that was not more than 5 percentage points and a decline that was not more than 10 percentage points at week 52) (Table S5 in the Supplementary Appendix).

ACUTE EXACERBATIONS

In INPULSIS-1, there was no significant difference between the nintedanib and placebo groups in the time to the first acute exacerbation (haz-

ard ratio in the nintedanib group, 1.15; 95% CI, 0.54 to 2.42; P=0.67) (Fig. 2A), and the proportion of patients with at least one investigator-reported acute exacerbation was similar in the nintedanib and placebo groups (6.1% and 5.4%, respectively). In INPULSIS-2, there was a significant increase in the time to the first acute exacerbation in the nintedanib group as compared with the placebo group (hazard ratio, 0.38; 95% CI, 0.19 to 0.77; P=0.005) (Fig. 2B), and the proportion of patients with at least one investigator-reported acute exacerbation was lower in the nintedanib group than in the placebo group (3.6% vs. 9.6%). In the prespecified pooled analysis, there was no significant difference between the nintedanib and placebo groups in time to first investigator-reported acute exacerbation (hazard ratio, 0.64; 95% CI, 0.39 to 1.05; P=0.08); the proportion of patients with at least one investigator-reported acute exacerbation was 4.9% in the nintedanib group and 7.6% in the placebo group (Fig. S4 and S5 in the Supplementary Appendix). A prespecified sensitivity analysis of pooled data on the time to the first adjudicated acute exacerbation (confirmed or suspected) showed that nintedanib had a significant benefit as compared with placebo (Table S6 and Fig. S6 in the Supplementary Appendix).

SGRQ SCORE

In INPULSIS-1, there was no significant between-group difference in the adjusted mean change in the total SGRQ score from baseline to week 52 (4.34 points in the nintedanib group and 4.39 points in the placebo group; difference, −0.05; 95% CI, −2.50 to 2.40; P=0.97); in INPULSIS-2, there was a significantly smaller increase in the total SGRQ score at week 52 (consistent with less deterioration in health-related quality of life) in the nintedanib group than in the placebo group (2.80 points vs. 5.48 points; difference, −2.69; 95% CI, −4.95 to −0.43; P=0.02) (Fig. S7A and S7B in the Supplementary Appendix).

In the prespecified pooled analysis of the total SGRQ score, there was no significant difference in the adjusted mean change from baseline to week 52 between the nintedanib and placebo groups (difference, −1.43 points; 95% CI, −3.09 to 0.23; P=0.09) (Fig. S7C in the Supplementary Appendix). Changes from baseline in SGRQ domain scores were consistent with the changes in the total SGRQ score in each trial (Tables S7A and S7B in the Supplementary Appendix) and in the pooled analysis (Table S7C in the Supplementary Appendix).

DEATHS

In the prespecified pooled analysis, there was no significant between-group difference in death from any cause, death from a respiratory cause, or death that occurred between randomization and 28 days after the last dose of the study drug (Table S8 in the Supplementary Appendix). The proportion of patients who died from any cause over the 52-week treatment period was 5.5% in the nintedanib group and 7.8% in the placebo group (hazard ratio in the nintedanib group, 0.70; 95% CI, 0.43 to 1.12; P=0.14) (Fig. S8 in the Supplementary Appendix).

ADVERSE EVENTS

The most frequent adverse event in the nintedanib groups in both trials was diarrhea (Table 3). Among the patients in the nintedanib groups who had diarrhea, most reported events that were of mild or moderate intensity (93.7% in INPULSIS-1 and 95.2% in INPULSIS-2). Diarrhea led to premature discontinuation of the study drug in 14 patients receiving nintedanib (4.5%) and none of the patients receiving placebo in INPULSIS-1 and in 14 patients receiving nintedanib (4.3%) and 1 receiving placebo (0.5%) in INPULSIS-2.

In both trials, the proportion of patients with serious adverse events was similar in the nintedanib and placebo groups (Table 3). In INPULSIS-1, serious adverse events were reported in 31.1% of patients in the nintedanib group and in 27.0% of patients in the placebo group; in INPULSIS-2, the percentages were 29.8% and 32.9%, respectively.

In both trials, a higher proportion of patients in the nintedanib groups than in the placebo groups had elevated levels of liver enzymes (Table S9 in the Supplementary Appendix). In INPULSIS-1, a total of 15 patients in the nintedanib group (4.9%) and 1 patient in the placebo group (0.5%) had levels of aspartate aminotransferase, alanine aminotransferase, or both that were three or more times the upper limit of the normal range. In INPULSIS-2, a total of 17 patients in the nintedanib group (5.2%) and 2 patients in the placebo group (0.9%) had such elevations.

Among the infrequent events (those occurring

Table 3. Adverse Events.

Event	INPULSIS-1		INPULSIS-2	
	Nintedanib (N=309)	Placebo (N=204)	Nintedanib (N=329)	Placebo (N=219)
	number of patients (percent)			
Any adverse event	298 (96.4)	181 (88.7)	311 (94.5)	198 (90.4)
Any adverse event, excluding progression of idiopathic pulmonary fibrosis*	296 (95.8)	179 (87.7)	311 (94.5)	197 (90.0)
Most frequent adverse events†				
Diarrhea	190 (61.5)	38 (18.6)	208 (63.2)	40 (18.3)
Nausea	70 (22.7)	12 (5.9)	86 (26.1)	16 (7.3)
Nasopharyngitis	39 (12.6)	34 (16.7)	48 (14.6)	34 (15.5)
Cough	47 (15.2)	26 (12.7)	38 (11.6)	31 (14.2)
Progression of idiopathic pulmonary fibrosis*	31 (10.0)	21 (10.3)	33 (10.0)	40 (18.3)
Bronchitis	36 (11.7)	28 (13.7)	31 (9.4)	17 (7.8)
Upper respiratory tract infection	28 (9.1)	18 (8.8)	30 (9.1)	24 (11.0)
Dyspnea	22 (7.1)	23 (11.3)	27 (8.2)	25 (11.4)
Decreased appetite	26 (8.4)	14 (6.9)	42 (12.8)	10 (4.6)
Vomiting	40 (12.9)	4 (2.0)	34 (10.3)	7 (3.2)
Weight loss	25 (8.1)	13 (6.4)	37 (11.2)	2 (0.9)
Severe adverse events‡	81 (26.2)	37 (18.1)	93 (28.3)	62 (28.3)
Serious adverse events‡	96 (31.1)	55 (27.0)	98 (29.8)	72 (32.9)
Fatal adverse events	12 (3.9)	10 (4.9)	25 (7.6)	21 (9.6)
Adverse events leading to treatment discontinuation§	65 (21.0)	22 (10.8)	58 (17.6)	33 (15.1)
Gastrointestinal disorders	26 (8.4)	3 (1.5)	21 (6.4)	2 (0.9)
Respiratory, thoracic, and mediastinal disorders	12 (3.9)	10 (4.9)	8 (2.4)	18 (8.2)
Investigation results¶	10 (3.2)	1 (0.5)	8 (2.4)	1 (0.5)
Cardiac disorders	5 (1.6)	4 (2.0)	2 (0.6)	3 (1.4)
General disorders and conditions involving site of study-drug administration‖	8 (2.6)	3 (1.5)	2 (0.6)	1 (0.5)

* Progression of idiopathic pulmonary fibrosis was defined in accordance with the definition of idiopathic pulmonary fibrosis in the *Medical Dictionary for Regulatory Activities*, version 16.1, which includes disease worsening and exacerbations of idiopathic pulmonary fibrosis.

† The most frequent adverse events were defined as those with an incidence of more than 10% in any study group.

‡ A severe adverse event was related to intensity and was defined as an event that was incapacitating or that caused an inability to work or to perform usual activities. A serious adverse event was defined as any adverse event that resulted in death, was immediately life-threatening, resulted in persistent or clinically significant disability or incapacity, required or prolonged hospitalization, was related to a congenital anomaly or birth defect, or was deemed serious for any other reason.

§ Adverse events leading to study-drug discontinuation were reported when they occurred in 2% or more of patients in any study group and are listed according to system organ class. The analysis included adverse events with an onset after administration of the first dose of study medication and up to 28 days after administration of the last dose.

¶ Investigation results refer to the results of clinical laboratory tests, radiologic tests, physical examination, and physiologic tests.

‖ These events include disorders or conditions that involve several body systems or sites, including chest pain, fatigue, asthenia, and general deterioration of physical health.

in less than 2% of a study group) that were of potential clinical importance, myocardial infarction was reported in 5 patients in the nintedanib group (1.6%) and 1 patient in the placebo group (0.5%) in INPULSIS-1, and in 5 patients in the nintedanib group (1.5%) and 1 patient in the placebo group (0.5%) in INPULSIS-2. In total, two events in the nintedanib groups and one event in

the placebo groups were fatal. Occurrences of adverse events related to cardiac disorders, including ischemic heart disease, are summarized in Table S10 in the Supplementary Appendix.

DISCUSSION

In both the INPULSIS trials, nintedanib significantly reduced the rate of decline in FVC over the 52-week treatment period. The robustness of this finding was supported by the results of all prespecified sensitivity analyses, including those assessing alternative ways of handling missing data. The treatment effect for the annual rate of decline in FVC was consistent with the treatment effect for the absolute change from baseline in FVC. The curves for changes from baseline in FVC over time in the nintedanib and placebo groups separated early in the two studies and continued to diverge over time.

A smaller proportion of patients in the nintedanib groups than in the placebo groups had an absolute decline in the percentage of predicted FVC of more than 5 percentage points, an observation that supports the clinical relevance of the results. No consistent effect of nintedanib on the time to the first acute exacerbation or on the change in the total SGRQ score was observed in the two trials. This difference in the key secondary end point results between INPULSIS-1 and INPULSIS-2 was not explained by the differences in baseline characteristics between the trials.

Acute exacerbations of idiopathic pulmonary fibrosis are events of major clinical significance that are associated with high morbidity and mortality.[17,19] The INPULSIS trials showed that the effect of nintedanib was inconsistent with respect to the risk of investigator-reported acute exacerbations. Exacerbations are relatively rare events in patients with idiopathic pulmonary fibrosis who are in clinical trials and are difficult to assess and categorize, which may explain some of the heterogeneity in our findings.[20]

In both trials, the most frequent adverse events in the nintedanib groups were gastrointestinal in nature, with the majority of patients who received nintedanib reporting diarrhea. However, the proportion of patients in the nintedanib groups with diarrhea that led to premature discontinuation of the study medication was less than 5% (4.5% in INPULSIS-1 and 4.3% in INPULSIS-2). In both trials, the mean dose intensity in the nintedanib groups was greater than 90%. These results show that although adverse events associated with nintedanib treatment were not infrequent, the dosing regimen used in the INPULSIS trials was successful in minimizing treatment discontinuations. Although serious adverse events reflecting ischemic heart disease were balanced between the nintedanib and placebo groups, a higher percentage of patients in the nintedanib groups had myocardial infarctions. The clinical significance of this finding is unknown, and further observation in larger cohorts is needed.

In conclusion, data from the INPULSIS trials show that in patients with idiopathic pulmonary fibrosis, nintedanib reduced the decline in FVC, which is consistent with a slowing of disease progression. There were significant differences in favor of nintedanib for the time to the first acute exacerbation and the change from baseline in the total SGRQ score in INPULSIS-2 but not in INPULSIS-1. Adverse events were common in the nintedanib groups in both trials; nonetheless, most patients continued to receive nintedanib for the duration of the treatment period.

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APPENDIX

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REFERENCES

1. Raghu G, Collard HR, Egan JJ, et al. An official ATS/ERS/JRS/ALAT statement — idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. Am J Respir Crit Care Med 2011; 183:788-824.
2. du Bois RM, Weycker D, Albera C, et al. Forced vital capacity in patients with idiopathic pulmonary fibrosis: test properties and minimal clinically important difference. Am J Respir Crit Care Med 2011;184: 1382-9.
3. Collard HR, King TE Jr, Bartelson BB, Vourlekis JS, Schwarz MI, Brown KK. Changes in clinical and physiologic variables predict survival in idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2003;168:538-42.
4. Flaherty KR, Mumford JA, Murray S, et al. Prognostic implications of physiologic and radiographic changes in idiopathic interstitial pneumonia. Am J Respir Crit Care Med 2003;168:543-8.
5. Latsi PI, du Bois RM, Nicholson AG, et al. Fibrotic idiopathic interstitial pneumonia: the prognostic value of longitudinal functional trends. Am J Respir Crit Care Med 2003;168:531-7.
6. King TE Jr, Safrin S, Starko KM, et al. Analyses of efficacy end points in a controlled trial of interferon-gamma1b for idiopathic pulmonary fibrosis. Chest 2005; 127:171-7.
7. King TE Jr, Pardo A, Selman M. Idiopathic pulmonary fibrosis. Lancet 2011; 378:1949-61.
8. Chaudhary NI, Roth GJ, Hilberg F, et al. Inhibition of PDGF, VEGF and FGF signalling attenuates fibrosis. Eur Respir J 2007;29:976-85.
9. Coward WR, Saini G, Jenkins G. The pathogenesis of idiopathic pulmonary fibrosis. Ther Adv Respir Dis 2010;4:367-88.
10. Wollin L, Maillet I, Quesniaux V, Holweg A, Ryffel B. Anti-fibrotic and anti-inflammatory activity of the tyrosine kinase inhibitor nintedanib in experimental models of lung fibrosis. J Pharmacol Exp Ther 2014;349:209-20.
11. Hilberg F, Roth GJ, Krssak M, et al. BIBF 1120: triple angiokinase inhibitor with sustained receptor blockade and good antitumor efficacy. Cancer Res 2008;68: 4774-82.
12. Richeldi L, Costabel U, Selman M, et al. Efficacy of a tyrosine kinase inhibitor in idiopathic pulmonary fibrosis. N Engl J Med 2011;365:1079-87.
13. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. Eur Respir J 2005;26:319-38.
14. Jones PW, Quirk FH, Baveystock CM. The St. George's Respiratory Questionnaire. Respir Med 1991;85:Suppl B:25-31.
15. Barr JT, Schumacher GE, Freeman S, LeMoine M, Bakst AW, Jones PW. American translation, modification, and validation of the St. George's Respiratory Questionnaire. Clin Ther 2000;22:1121-45.
16. Jones PW. St. George's Respiratory Questionnaire: MCID. COPD 2005;2:75-9.
17. Collard HR, Moore BB, Flaherty KR, et al. Acute exacerbations of idiopathic pulmonary fibrosis. Am J Respir Crit Care Med 2007;176:636-43.
18. Cotes JE, Chinn DJ, Quanjer PH, Roca J, Yernault JC. Standardization of the measurement of transfer factor (diffusing capacity). Report of the Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official statement of the European Respiratory Society. Eur Respir J Suppl 1993;16:41-52.
19. Song JW, Hong SB, Lim CM, Koh Y, Kim DS. Acute exacerbation of idiopathic pulmonary fibrosis: incidence, risk factors and outcome. Eur Respir J 2011;37:356-63.
20. Collard HR, Yow E, Richeldi L, Anstrom KJ, Glazer C. Suspected acute exacerbation of idiopathic pulmonary fibrosis as an outcome measure in clinical trials. Respir Res 2013;14:73.

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LYMPHATIC MANIFESTATIONS OF LYMPHANGIOLEIOMYOMATOSIS

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ABSTRACT

Lymphangioleiomyomatosis (LAM) is a slowly progressive, low grade, metastasizing neoplasm, associated with cellular invasion and cystic destruction of the pulmonary parenchyma. Although the source of LAM cells that infiltrate the lung is unknown, available evidence indicates that the disease spreads primarily through lymphatic channels, often involving abdominal, axial, and retroperitoneal nodes, suggestive of an origin in the pelvis. LAM cells harbor mutations in tuberous sclerosis genes and produce lymphangiogenic growth factors, which facilitate access to and movement through the lymphatic system and likely play an important role in destructive tissue remodeling in the lung. Lymphatic manifestations of LAM include thoracic duct wall invasion, lymphangioleiomyoma formation, chylous fluid collections in the peritoneal, pleural, and pericardial spaces, chyloptysis, chylocolporrhea/chylometrorrhea, chyle leak from the umbilicus, chylous pulmonary congestion, and lower extremity lymphedema. LAM lesions express lymphangiogenic growth factors VEGF-C and VEGF-D; growth factor receptors, VEGFR-2 and VEGFR-3; and markers LYVE-1 and podoplanin, and are laced with chaotic lymphatic channels. Serum VEGF-D is elevated in 70% of patients with LAM and is a clinically useful diagnostic and prognostic biomarker. Molecular targeted therapy with sirolimus stabilizes lung function, is anti-lymphangiogenic, and is highly effective for the lymphatic and chylous complications of LAM. Future trials in patients with LAM who have lymphatic manifestations or elevated serum VEGF-D will likely focus on the VEGF-C/VEGF-D/VEGFR-3 axis.

Keywords: lymphangioleiomyomatosis (LAM), sporadic LAM, tuberous sclerosis LAM, vascular endothelial growth factors (VEGF), angiomyolipoma (AML), sirolimus (Rapamycin)

Lymphangioleiomyomatosis is an uncommon systemic neoplasm targeting women that typically presents in the third decade of life with cystic destruction of the lung associated with dyspnea on exertion and pneumothorax (1). Respiratory limitations in LAM are most commonly caused by airflow limitation and destruction of the pulmonary capillary bed leading to a reduction in diffusing capacity (2). Less common initial pulmonary symptoms include hemoptysis, cough, and chest pain. Patients with LAM may have renal, hepatic or splenic angiomyolipomas (fat containing benign tumors with aneurysmal vessels that are

prone to bleeding). Lymphatic manifestations are present in a substantial fraction of patients with LAM, including chylous pleural effusions in 30%, lymphangioleiomyomas in the abdomen, pelvis, or mediastinum in 29%, chylous ascites in 10%, and lower extremity lymphedema in 4%. Lymphatic fistulas can lead to loss of chyle into other potential spaces, hollow viscera, or the environment, producing manifestations of chylous pericardial effusion, chyloptysis, plastic bronchitis, chylocolporrhea (chylometrorrhea), chyluria, and leakage of chyle from the umbilicus. Communication between the intestinal tract and lymphatic system can lead to protein losing enteropathy or lymphatic sepsis and death. Prolonged external drainage of chyle or repeated taps of chylous accumulations in the chest and abdomen can result in nutritional and trace element deficiencies. Serum VEGF-D is elevated in about 70% of LAM patients, especially those who have known lymphatic involvement (3).

Molecular Genetics and Epidemiology of LAM

LAM is caused by mutations in tuberous sclerosis complex (TSC) genes, TSC1, or TSC2 (4). TSC is an autosomal dominant tumor suppressor syndrome with variable penetrance that usually presents in childhood with seizures, skin lesions, and benign hamartomatous tumors of brain, heart, and kidney (5). LAM can occur in patients with TSC (TSC-LAM), and also sporadically in patients who do not have any inherited genetic disease (sporadic LAM or S-LAM). Patients with TSC-LAM have germ line mutations in tuberous sclerosis genes, most often (66% of the time) acquired during embryogenesis rather than inherited from a parent, and develop tumors in locations where second somatic mutations or 'hits' occur (6). Sporadic LAM, in contrast, is thought be due to the occurrence of two somatic hits in TSC2 (4).

Women with TSC develop pulmonary cystic changes in an age-dependent manner, affecting about 22% of patients by the age of 20 years, and up to 80% by the age of 40 years (7). Only a fraction of women with TSC who have cystic changes on HRCT develop pulmonary symptoms, however, perhaps as low as 5-10%. Although up to 10-15% of men with TSC may have cystic changes on HRCT, symptomatic LAM in men is extremely rare (8). It should be noted that not all cystic changes in patients with TSC are due to LAM; biopsies in some cases have shown atypical HMB-45 positive lesions or no evidence of smooth muscle cell infiltration that is characteristic of LAM (9), so studies of LAM prevalence in patients with TSC that rely solely on radiographic changes may overestimate the numbers of patients affected. The global prevalence of TSC is about 1 million people, about half of whom are women. A conservative estimate is that 40-50% of adult females with TSC have cysts consistent with TSC-LAM, suggesting a worldwide prevalence of about 200,000 to 250,000 affected or about 1 per 20,000 persons. S-LAM, in contrast, is thought to affect about 1 in 200,000 persons (10,11).

Molecular Pathogenesis of LAM

LAM can be caused by mutations in either of the tuberous sclerosis genes, TSC1 or TSC2, which encode the proteins hamartin and tuberin, respectively. These proteins form a complex that negatively regulates mTOR activity through an intermediate called Ras Homologue Enriched in Brain (Rheb) (12,13). Defects or deficiencies of TSC1 or TSC2 result in constitutive mTOR activation and dysregulated protein translation and cellular proliferation, autophagy and survival *(Figs. 1 and 2)* (14-16). Estradiol promotes the proliferation of TSC2 deficient cells in rat models of TSC-LAM (17) perhaps by enhancing the expression of Fra1 (18).

The cells that infiltrate and destroy the lung in LAM arise from an unknown source. They migrate to the lung and form nodular and cystic lesions in the interstitial spaces,

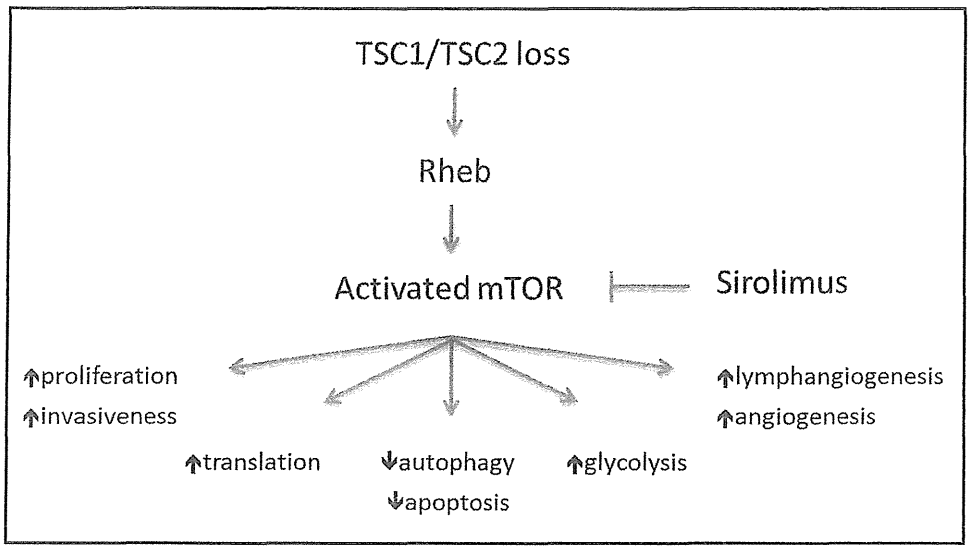

Fig. 1. Signaling networks in LAM cells. TSC1 and TSC2 form a complex that integrates input from upstream signaling cascades, such as those emanating from membrane tyrosine kinase receptors. Rheb, which is normally suppressed by TSC1/TSC2, becomes activated when TSC1 or TSC2 are defective or deficient. Rheb activates mTOR leading to multiple cellular functions that confer a cancer-like phenotype on the LAM cell. Sirolimus binds to FKBP12 and stearically inhibits mTOR actions.

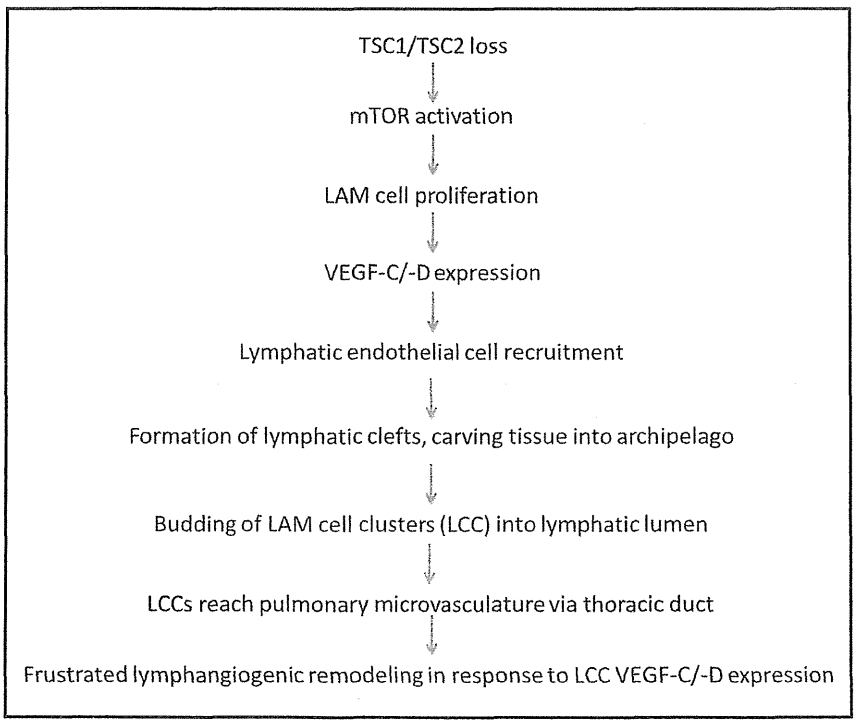

Fig. 2. Role of TSC mutations and lymphatic processes in the pathogenesis of LAM.

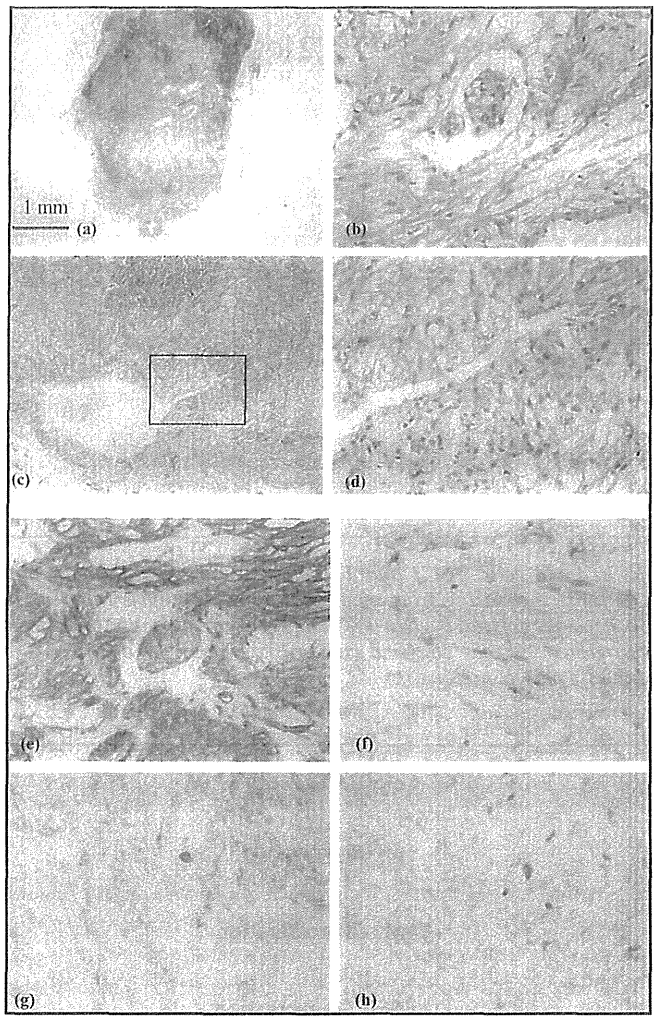

Fig. 3. (a-h) LAM histology and immunohistochemical staining. a) Involvement of a lymph node by LAM. About 70% of this lymph node was replaced by bundles of immature smooth muscle cells (H&E stain, x1). Higher magnifications (a) revealed two LAM cell clusters in a lymphatic vessel within the lymph node (b; H&E stain, x40) and LAM cell proliferation in the wall of a lymphatic vessel (c; H&E stain, x10. d; H&E stain, x40 of selected area). Immunohistochemical staining of the LAM lesion with anti-alpha-smooth muscle actin (e) highlighted bundles of LAM cells and a LAM cell cluster within the lymphatic vessel (magnification x60). Immunohistochemical staining of a LAM lesion with HMB-45 (f) stained the cytoplasm of LAM cells (magnification x60) while staining of the LAM lesion with anti-progesterone receptor (g) and -estrogen receptor (h) antibodies were positive in a nuclear pattern (magnification for both x60).

enveloping and sometimes invading lymphatics, airways, and blood vessels. LAM lesions are composed of chaotically arranged epithelioid and spindle shaped smooth muscle cells that stain with antibodies against smooth muscle actin, estrogen receptors, progesterone receptors and gp-100 (HMB-45), and other melanocytic proteins (19) (*Fig. 3*). Lymphangiogenic growth factors, VEGF-C, VEGF-D, are also expressed in the LAM lesion (20), most likely through hypoxia inducible factor (HIF) or HIF-related pathways.

VEGF-C and -D are ligands for VEGFR-3, a receptor that exhibits a highly restricted expression pattern in lymphatic endothelial cells and fenestrated blood vessels found in endocrine organs such as pancreas, thyroid, and adrenal glands (21-24). Other lymphatic markers that are present in the LAM lesion are podoplanin and LYVE-1 (20). Cleft like spaces that are lined with VEGFR-3 expressing lymphatic endothelial cells are often found within both pulmonary and extrapulmonary LAM lesions (20). Cyst formation in the lung may be the result of indiscriminate expression of matrix degrading enzymes known to be expressed in LAM lesions including MMP-2, MMP-9, and Cathepsin K, or may be a form of 'frustrated lymphangiogenesis,' a term coined to describe chaotic remodeling process occurring in response to lymphangiogenic signals that were appropriate during development but 'confusing' to a mature organ (*Fig. 2*). Cysts are often but not always bordered by crescentic, non-circumferential collections of smooth muscle cells, often with a partial interior lining of hypertrophic alveolar epithelial cells.

Candidates for the primary tumor site in LAM include the bone marrow, angiomyolipoma, lymphatic tree, or the uterus (25,26). Some believe the cell of origin may be the pericyte, whereas others believe that like other PEComas, there is no anatomically normal counterpart for LAM cells; i.e., that their unique phenotype is driven by dysregulated cellular signaling. Circulating LAM cells are present in the blood (27) and lymphatic fluids (28-30). Lymph node involvement is typically restricted to the axial distribution, most often in an ascending gradient pattern from the pelvis and lower abdomen. Mediastinal and hilar lymph nodes may also be involved, but only a few case reports of peripheral lymph node involvement have appeared in the literature. Lymphangioleiomyomas are dilated, fluid filled lymphatic neoplasms that occur in the abdomen and pelvis of patients with LAM (31,32). Because of their hypodense centers, they can be easily confused with lymphomas, or partially necrotic genitourinary malignancies such as ovarian or uterine cancer.

Clinical Presentation

LAM typically presents with progressive dyspnea on exertion or recurrent pneumothorax, or can be discovered incidentally on CT scans obtained for another purpose. The presentation mimics asthma and chronic obstructive pulmonary disease and the diagnosis is often delayed. The average number of pneumothoraces prior to diagnosis of LAM in the USA is 2.2 (33,34). Angiomyolipomas (AMLs) are present in about 30% of patients with S-LAM and about 80% of patients with TSC-LAM. Chylous pleural effusions *(Fig. 4)* or chylous pulmonary congestion *(Figs. 4,5)* are also seen in about 30% of S-LAM patients, and less frequently in patients with TSC-LAM.

High resolution computed tomography of the chest is the most sensitive diagnostic modality for LAM. Characteristic findings include thin-walled cysts with discrete borders, ranging from a few mm to a few cm in diameter, diffusely distributed throughout the lung. The spaces between cysts are often comprised of radiographically normal appearing pulmonary parenchyma , although recent textural analyses suggest that paracystic lung tissue in LAM may not be truly normal (35).

Pulmonary function tests are often normal in the early stages of LAM, when few cysts are present. Approximately 34% of patients enrolled in the NHLBI registry had normal spirometry (36). The earliest changes in LAM are often a reduction in diffusing capacity and an increase in residual volume. Over time, most patients with LAM develop progressive obstructive ventilatory abnormalities, including a reduction in FEV1 and the FEV1/FVC ratio. Lung volumes can also become abnormal; in the NHLBI registry 11% of patients had a restrictive defect and 6% of patients had hyperinflation (36).

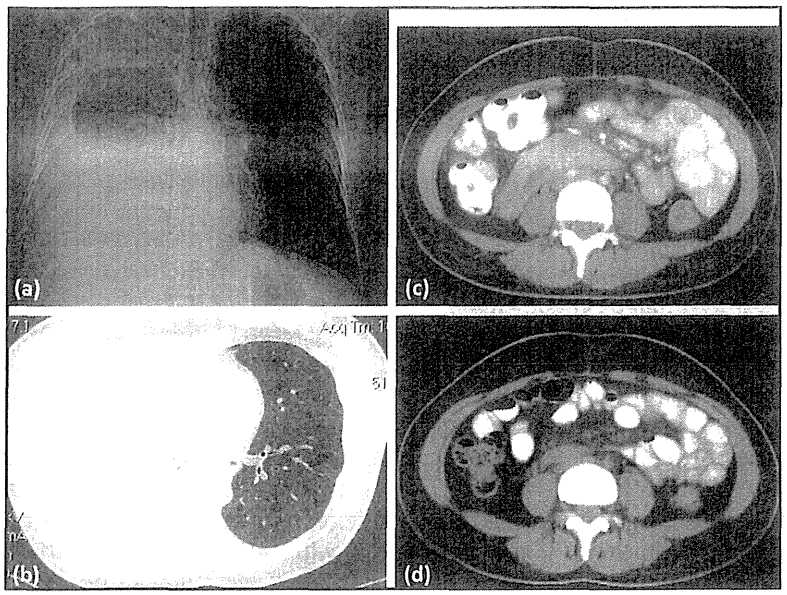

Fig. 4. Chest radiograph (a) and HRCT (b) images of a 37 year old female with a right-sided chylous pleural effusion and cystic changes due to LAM. CT of the abdomen (c) revealed a retroperitoneal mass with hypodense center that was associated with a protein losing enteropathy, suggesting communication with the gut. Needle aspiration showed HMB-45 positive cells consistent with LAM. 1 year of Sirolimus therapy resulted in resolution of retroperitoneal mass (d).

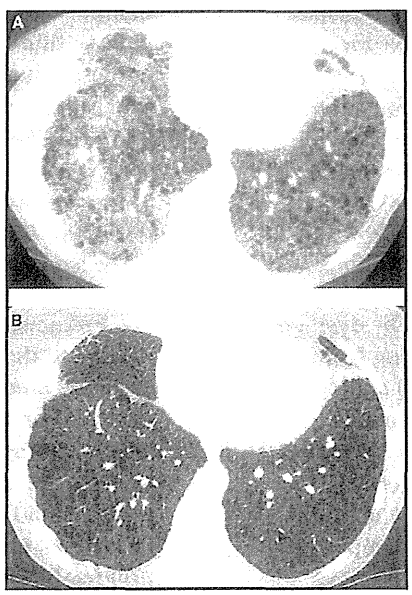

Fig. 5: Pulmonary lymphatic congestion (A) due to chylous reflux is a cause of worsening dyspnea and hypoxemia in LAM. Note the diffuse reticular and ground glass densities. Treatment with Sirolimus has been demonstrated to improve the congestion (B). Reprinted with permission of the American Thoracic Society from Moua et al (2012): Resolution of Chylous Pulmonary Congestion and Respiratory Failure in Lymphangioleiomyomatosis with Sirolimus Therapy. Am. J. of Resp. Crit. Care Med. 186(4): page 390.

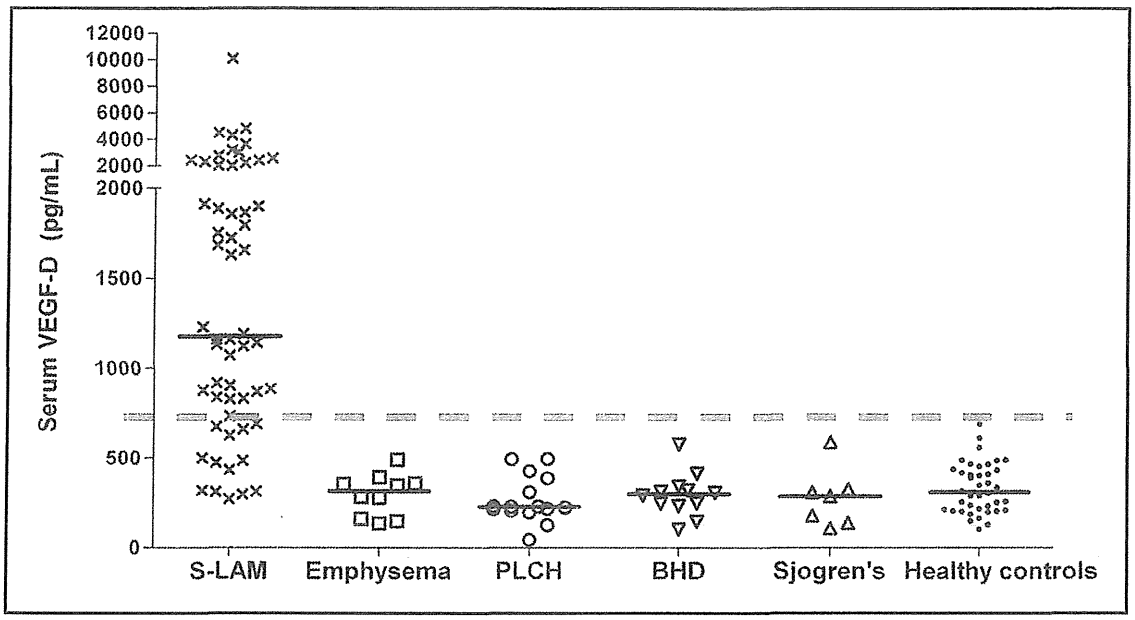

Fig. 6. A serum VEGF-D level >800pg/ml differentiates sporadic LAM (S-LAM) from other cystic diseases of the lung, including emphysema, pulmonary Langerhans cell histiocytosis (PLCH), Birt Hogg Dube (BHD) Syndrome, and Sjogren's cystic lung disease. (Modified with permission from Young et al) (65)

Diagnosis

The average age at the time of diagnosis of LAM is 35 years, although it has been described in the young and the old, including a 12 year old girl (37) and in an 86 year old woman (38). A clinical diagnosis of LAM can be based on the European Respiratory Society Guidelines (39), without the need for tissue confirmation in some cases. In a patient with typical cystic changes on HRCT, the presence of any one of the following additional features are diagnostic by ERS Criteria; tuberous sclerosis, chylothorax, lymphangiomyoma or an angiomyolipoma. We maintain that a serum VEGF-D >800 pg/ml is also diagnostic when the CT is characteristic (40) (*Fig. 6*). When tissue is required, transbronchial biopsy has a yield of >50% and appears to be safe in several small series (41,42). Video-assisted thoracoscopic biopsy remains the gold standard, but is required for diagnosis in only about 15-20% of cases when all of the above modalities are employed, and should be reserved for cases where less invasive approaches fail. Cystic lung diseases that are often considered in the differential of LAM include emphysema, alpha-1 antitrypsin deficiency, pulmonary Langerhans cell histiocytosis, Birt-Hogg-Dubé syndrome, or cystic lung disease due to follicular bronchiolitis, lymphocytic interstitial pneumonitis (as occurs in Sjögren's syndrome), hypersensitivity pneumonitis or desquamative interstitial pneumonitis. Serum VEGF-D testing can be useful for making the diagnosis of LAM when positive (>800 pg/ml) (*Fig. 6*), but is not informative when negative. It is often helpful to obtain an alpha 1-antitrypsin protein level, SS-A, SS-B, ANA, RA, and WESR to help with differential diagnosis. Cytological analysis of cells obtained from pleural effusion or ascitic fluid can be diagnostic (29). Pathological examination of tissue obtained by transbronchial or VATSs biopsy includes staining for HMB-45,

an antibody that recognizes the gp-100 protein in the melanogenesis pathway. HMB-45 staining is specific for LAM but it can be sparse and may be even absent. Other markers that stain positive in LAM tissue include alpha-smooth muscle actin, desmin, vimentin, hormone receptors (ER,PR), VEGF-R3, podoplanin, among many others (19).

Involvement of Lymphatics

Approximately 30% of patients with LAM have axial abdominal or thoracic lymphadenopathy, compared to about 9% of patients TSC-LAM. LAM can occasionally be restricted to the retroperitoneum, abdomen, or pelvis, with a normal HRCT or only scattered rare lung cysts, consistent with regional spread from a pelvic or low abdominal source. More often, abdominal LAM manifestations occur in patients who have diffuse cystic change on CT.

Clusters of LAM cells in the chylous pleural fluid of patients with LAM were first described by Valensi in 1973 (43). Later, Itami and coworkers demonstrated that the clusters were also present within the dilated lymphatic circulation and were composed of alpha smooth muscle actin-positive spindle cells enveloped by a single layer of endothelial cells (44). They suggested that LAM cell clusters could be used diagnostically to obviate the need for biopsy in patients with chylous manifestations of LAM. More recent data from Japan provided additional evidence that a likely source and mechanism of spread of LAM may be through the lymphatic circulation. In a small autopsy series, Kumasaka and colleagues described the infiltration of the thoracic duct wall and surrounding fat by LAM cells (30). They also noted the presence of LAM cell clusters enveloped by lymphatic endothelial cells budding from the walls of lymphatic vessels and in the lumen of lymphatic channels and the thoracic duct. LAM cell clusters were found in chylous pleural and peritoneal effusions and within lymphatic vessels.

Lymphangioleiomyomas in LAM most commonly affect the retroperitoneal and pelvic regions (30,36,45-48). On CT screening, lymphangioleiomyomas appear as well circumscribed, lobulated, low density cystic to solid masses of various sizes (31). Diurnal variation in size and echotexture of lymphangioleiomyomas occurs, suggesting gravitational and dietary influences on the retention of lymphatic fluid in these lesions (31), with increase in size between morning and afternoon.

Chyle leakage into body cavities occurs in a subset of LAM patients, due to direct invasion or proximal obstruction of the lymphatic system, particularly the thoracic duct and its tributaries (30,49). Chylothorax (36,50), chylous ascites, chyle leak in pericardial space (51), chyluria (52,53), chyle loss in intestinal lumen (54-55) and also chyle loss in vagina has been described in various studies and case reports (33,49). Chyloptysis can occur with development of a bronchopleural fistula (56). Chylous bronchial casts have been described in case reports (57). About 10-15% of LAM patients have chylothorax at presentation, eventually affecting 20-40% of patients at some point in the disease course (45,48,58,59). In most cases, fluid accumulation is likely the result of chylous reflux, increased pressures in the lymphatic vessels of the lung and retrograde weeping of chyle from visceral and diaphragmatic pleural surfaces (60). Transdiaphragmatic flow of chylous ascites through porous defects in the diaphragm may also result in chylothorax, most commonly on the right. Chyle loss in intestine or protein-losing enteropathy may occur (55) due to retroperitoneal involvement by LAM and associated intestinal lymphangiectasia. Patients may present with diarrhea, peripheral edema, and hypoalbuminemia (34,59). Lymphedema with chyluria was first described in 1968 (61,62). Lymphedema has been described in a case report with LAM without evidence of pulmonary involvement (63).

Lymphatic Biomarkers in LAM

Lymphatic growth factors have shown to have diagnostic, prognostic and predictive utility in patients with LAM. Serum VEGF-D, but not VEGF-C or VEGF-A, is elevated in serum of patients with LAM. There is a negative correlation between serum VEGF-D and markers of disease severity such as FEV1/FVC and diffusion capacity of lung for carbon monoxide (DLCO) (3). A statistically significant correlation between greater lymphatic involvement and higher VEGF-C expression by immunohistochemistry has been demonstrated, and both VEGF-C and -D (by IHC) were associated with worse prognosis and more rapid progression, based on the LAM histology score (LHS) and time to death or transplantation (20). Young et al demonstrated that VEGF-D levels are significantly elevated in patients with LAM compared to those with other cystic lung diseases, such as those due to PLCH, Sjögren's cystic lung disease, and emphysema, and can obviate the need for lung biopsy in patients with typical cystic change on HRCT (40,64,65). Furthermore, VEGF-D levels were much higher in women with TSC and LAM than in women with TSC and normal HRCT (40,65). Glasgow et al also showed that VEGF-D levels appear to reflect lymphatic involvement. Patients with LAM and lymphatic involvement have significantly decreased pulmonary function (3). VEGF-D has also recently been shown to correlate with disease progression and treatment response, in that patients with higher levels are more likely to progress and more likely to respond to therapy with sirolimus (66).

Treatment

Sirolimus, also called rapamycin, blocks mTOR activation (*Fig. 1*) and partially restores homeostasis in cells with defective TSC gene function (67). The double blind, randomized Multicenter International LAM Efficacy of Sirolimus (MILES) trial, demonstrated that treatment with sirolimus for one year stabilized FEV1, reduced serum VEGF-D, and improved FVC, quality of life and functional performance. Sirolimus therapy has also been shown to be highly effective for the treatment of chylous effusions and lymphangioleiomyomas (68) (*Figs. 4d,5B*).

The management of chylous complications in patients with LAM is often challenging. Thoracentesis or paracentesis is indicated for relief of shortness of breath, but repeated chyle drainage can result in malnutrition and immunodeficiencies (69,70). Institution of a fat restricted diet enriched in medium chain triglycerides has been met with variable success in small studies (71). Peritoneovenous shunts have been used for management of refractory chylous ascites (69). Mechanical abrasion and chemical pleurodesis are generally effective therapies for chylothorax (50), but can result in diversion of flow and appearance of chylous fluid collection or drainage in other sites. Octreotide and other somatostatin analogues reduce lymphatic flow and have shown promise for the treatment of chylous effusions in other conditions (72-75) and use in LAM has been trialed (ClinicalTrials.gov Identifier: NCT00005906) and reported (76). Older studies of hormonal treatment with the progesterones or gonadotropin-releasing hormone (GnRH) suggested a salutary effect on chylous effusions in LAM (58). However, many conflicting reports regarding the effects of antiestrogen therapies in LAM have been published. Lymphedema can be managed with leg elevation, compressive hose, physiotherapy, and/or exercise (77).

Future Prospects

Anti-lymphangiogenic strategies are promising in LAM. mTOR inhibitors such as sirolimus and everolimus are anti-angiogenic and anti-lymphangiogenic, and effective in the treatment of chylous complications in LAM. Other candidates include tyrosine

kinase inhibitors such as axitinib, and pazopanib, anti-VEGF-D and anti-VEGF-C antibodies, and anti-VEGFR3 and soluble VEGFR3 have all been mentioned in the context of future trials. Stratification by VEGF-D status and menopausal state may greatly reduce the number of patients required for trials.

REFERENCES

1. Henske, EP, FX McCormack: Lymphangioleiomyomatosis - a wolf in sheep's clothing. J. Clin. Inv. 122 (2012), 3807-3816.
2. Crausman, RS, CA Jennings, RL Mortenson, et al: Lymphangioleiomyomatosis: The pathophysiology of diminished exercise capacity. Am. J. Respir. Crit. Care Med. 153 (1996), 1368-1376.
3. Glasgow, CG, NA Avila, J-P, Lin, et al: Serum vascular endothelial growth factor-d levels in patients with lymphangioleiomyomatosis reflect lymphatic involvement. Chest 135 (2009), 1293-1300.
4. Carsillo, T, A Astrinidis, EP Henske: Mutations in the tuberous sclerosis complex gene TSC2 are a cause of sporadic pulmonary lymphangioleiomyomatosis. Proc. Natl. Acad. Sci. USA 97 (2000), 6085-6090.
5. Crino, PB, KL Nathanson, EP Henske: The tuberous sclerosis complex. N. Engl. J. Med. 355 (2006), 1345-1356.
6. Knudson, AG: Two genetic hits (more or less) to cancer. Nat. Rev. Cancer 1 (2001), 157-162.
7. Cudzilo, CJ, RD Szczesniak, AS Brody, et al: Lymphangioleiomyomatosis screening in women with tuberous sclerosis. Chest 144 (2013), 578-585.
8. Aubry, M-C, JL Myers, JH Ryu, et al: Pulmonary lymphangioleiomyomatosis in a man. Am. J. Respir. Crit. Care Med. 162 (2000), 749-752.
9. Wenaden, AE, SJ Copley: Unilateral lymphangioleiomyomatosis. J. Thorac. Imaging 20 (2005), 226-228.
10. Harknett, EC, WY Chang, S Byrnes, et al: Use of variability in national and regional data to estimate the prevalence of lymphangioleiomyomatosis. QJM 104 (2011), 971-979.
11. O'Callaghan, FJ, AW Shiell, JP Osborne, et al: Prevalence of tuberous sclerosis estimated by capture-recapture analysis. Lancet 351(1998), 1490.
12. Inoki, K, Y Li, T Xu, et al: Rheb GTPase is a direct target of TSC2 GAP activity and regulates mTOR signaling. Genes Develop. 17 (2003), 1829-1834.
13. Castro, AF, JF Rebhun, GJ Clark, et al: Rheb binds tuberous sclerosis complex 2 (TSC2) and promotes S6 kinase activation in a rapamycin- and farnesylation-dependent manner. J. Biol. Chem. 278 (2003), 32493-32496.
14. Parkhitko, A, F Myachina, TA Morrison, et al: Tumorigenesis in tuberous sclerosis complex is autophagy and p62/sequestosome 1 (SQSTM1)-dependent. Proc. Natl. Acad. Sci. USA (2011), 12455-12460.
15. Malhowski, AJ, H Hira, S Bashiruddin, et al: Smooth muscle protein-22-mediated deletion of Tsc1 results in cardiac hypertrophy that is mTORC1-mediated and reversed by rapamycin. Hum. Molec. Gen. 20 (2011), 1290-1305.
16. Neuman, NA, EP Henske: Non-canonical functions of the tuberous sclerosis complex-Rheb signalling axis. EMBO Molec. Med. 3 (2011), 189-200.
17. Yu, JJ, VA Robb, TA Morrison, et al: Estrogen promotes the survival and pulmonary metastasis of tuberin-null cells. Proc. Natl. Acad. Sci. USA (2009), 2635-2640.
18. Gu, X, JJ Yu, D Ilter, et al: Integration of mTOR and estrogen-ERK2 signaling in lymphangioleiomyomatosis pathogenesis. Proc. Natl. Acad. Sci. USA 110 (2013), 14960-14965.
19. Matsumoto, Y, K Horiba, J Usuki, et al: Markers of cell proliferation and expression of melanosomal antigen in lymphangioleiomyomatosis. Am. J. Respir. Cell Molec. Biol. 21 (1999), 327-336.
20. Kumasaka, T, K Seyama, K Mitani, et al: Lymphangiogenesis in lymphangioleiomyomatosis: Its implication in the progression of lymphangioleiomyomatosis. Am. J. Surg. Pathol. 28 (2004), 1007-1016.
21. Tammela, T, B Enholm, K Alitalo, et al: The biology of vascular endothelial growth factors. Cardiovasc. Res. 65 (2005), 550-563.
22. Alitalo, K, T Tammela, TV Petrova: Lymphangiogenesis in development and human disease. Nature 438 (2005), 946-953.
23. Karpanen, T, K Alitalo: Molecular biology and pathology of lymphangiogenesis. Ann. Rev. Pathol. 3 (2008), 367-397.
24. Achen, MG, BK McColl, SA Stacker: Focus on lymphangiogenesis in tumor metastasis. Cancer Cell 7 (2005), 121-127.
25. Hayashi, T, T Kumasaka, K Mitani K, et al: Prevalence of uterine and adnexal involvement in pulmonary lymphangioleiomyomatosis: A clinicopathologic study of 10 patients. Am. J. Surg. Pathol. 35 (2011), 1776-1785.

26. Yu, J, A Astrinidis, EP Henske: Chromosome 16 loss of heterozygosity in tuberous sclerosis and sporadic lymphangiomyomatosis. Am. J. Resp. Crit. Care Med. 164 (2001), 1537-1540.
27. Cai, X, G Pacheco-Rodriguez, M Haughey, et al: Sirolimus decreases circulating lymphangioleiomyomatosis cells in patients with lymphangioleiomyomatosis. Chest 145 (2014), 108-112.
28. Crooks, DM, G Pacheco-Rodriguez, RM DeCastro, et al: Molecular and genetic analysis of disseminated neoplastic cells in lymphangioleiomyomatosis. Proc. Natl. Acad. Sci. USA 101 (2004), 17462-17467.
29. Hirama, M, R Atsuta, K Mitani, et al: Lymphangioleiomyomatosis diagnosed by immunocytochemical and genetic analysis of lymphangioleiomyomatosis cell clusters found in chylous pleural effusion. Int. Med. (Tokyo, Japan). 46 (2007), 1593-1596.
30. Kumasaka, T, K Seyama, K Mitani, et al: Lymphangiogenesis-mediated shedding of LAM cell clusters as a mechanism for dissemination in lymphangioleiomyomatosis. Am. J. Surg. Pathol. 29 (2005), 1356-1366.
31. Avila, NA, AJ Dwyer, DV Murphy-Johnson, et al: Sonography of lymphangioleiomyoma in lymphangioleiomyomatosis: Demonstration of diurnal variation in lesion size. AJR Am. J. Roentgenol.184 (2005), 459-464.
32. Avila, NA, J Bechtle, AJ Dwyer, et al: Lymphangioleiomyomatosis: CT of diurnal variation of lymphangioleiomyomas. Radiol. 221 (2001), 415-421.
33. Almoosa, KF, JH Ryu, J Mendez, et al: Management of pneumothorax in lymphangioleiomyomatosis: Effects on recurrence and lung transplantation complications. Chest 129 (2006), 1274-1281.
34. Johnson, SR, AE Tattersfield: Clinical experience of lymphangioleiomyomatosis in the UK. Thorax 55 (2000), 1052-1057.
35. Yao, J, AM Taveira-DaSilva, TV Colby, et al: CT grading of lung disease in lymphangioleiomyomatosis. AJR Am. J. Roentgenol. 199 (2012), 787-793.
36. Ryu, JH, J Moss, GJ Beck, et al: The NHLBI lymphangioleiomyomatosis registry: Characteristics of 230 patients at enrollment. Am. J. Resp. Crit. Care Med. 173 (2006), 105-111.
37. Nagy, B, Z Nabrady, Z Nemes: Pulmonary lymphangiomyomatosis in a preadolescent girl. N. Engl. J. Med. 338 (1998), 473-474.
38. Ho, TB, JH Hull, NC Hughes: An 86-year-old female with lymphangioleiomyomatosis. Eur. Resp. J. 28 (2006), 1065.
39. Johnson, SR, JF Cordier, R Lazor, et al: European Respiratory Society guidelines for the diagnosis and management of lymphangioleiomyomatosis. Eur. Resp. J. 35 (2010), 14-26.
40. Young, LR, Y Inoue, FX McCormack: Diagnostic potential of serum VEGF-D for lymphangioleiomyomatosis. N. Engl. J. Med. 358 (2008), 199-200.
41. Bonetti, F, PL Chiodera, M Pea, et al: Transbronchial biopsy in lymphangiomyomatosis of the lung. HMB45 for diagnosis. Am. J. Surg. Pathol. 17 (1993), 1092-1102.
42. Meraj, R, KA Wikenheiser-Brokamp, LR Young, et al: Utility of transbronchial biopsy in the diagnosis of lymphangio leiomyomatosis. Frontiers Med. 6 (2012), 395-405.
43. Valensi, QJ: Pulmonary lymphangiomyoma, a probable forme frust of tuberous sclerosis. A case report and survey of the literature. Am. J. Resp. Dis. 108 (1973), 1411-1415.
44. Itami, M, S Teshima, Y Asakuma, et al: Pulmonary lymphangiomyomatosis diagnosed by effusion cytology. A case report. Acta Cytol. 41 (1997), 522-528.
45. Urban, T, R Lazor, J Lacronique, et al: Pulmonary lymphangioleiomyomatosis. A study of 69 patients. Groupe d'Etudes et de Recherche sur les Maladies "Orphelines" Pulmonaires (GERM"O"P). Med. 78 (1999), 321-337.
46. Avila, NA, CC Chen, SC Chu, et al: Pulmonary lymphangioleiomyomatosis: Correlation of ventilation-perfusion scintigraphy, chest radiography, and CT with pulmonary function tests. Radiol. 214 (2000), 441-446.
47. Matsui, K, A Tatsuguchi, J Valencia, et al: Extrapulmonary lymphangioleiomyomatosis (LAM): Clinicopathologic features in 22 cases. Hum. Pathol. 31 (2000), 1242-1248.
48. Hayashida, M, K Seyama, Y Inoue, et al: The epidemiology of lymphangioleiomyomatosis in Japan: A nationwide cross-sectional study of presenting features and prognostic factors. Respirol. 12 (2007), 523-530.
49. Lam, B, GC Ooi, MP Wong, et al: Extrapulmonary presentation of asymptomatic pulmonary lymphangioleiomyomatosis. Respirol. 8 (2003), 544-547.
50. Ryu, JH, CH Doerr, SD Fisher, et al: Chylothorax in lymphangioleiomyomatosis. Chest 123 (2003), 623-627.
51. Hovland, A, H Bjornstad: Pericardial effusion in a patient with lymphangioleiomyomatosis. Scand. J. Inf. Dis. 36 (2004), 521-522.
52. Gray, SR, CB Carrington, JL Cornog, Jr: Lymphangiomyomatosis: Report of a case with ureteral involvement and chyluria. Cancer 35 (1975), 490-498.

53. Iwamoto, I, T Fujino, T Douchi: Chylous vaginal discharge in a patient with lymphangioleiomyomatosis. Am. J. Obstet. Gynec. 199 (2008), e5-6.
54. Lin, CC, TC Lee, KL Liu, et al: Education and imaging. Gastrointestinal: Lymphangioleiomyomatosis with protein-losing enteropathy. J. Gastro. Hepatol. 21 (2006), 1860.
55. Kinoshita, A, I Odagi, Y Aoki, et al: [Case of lymphangioleiomyomatosis associated with protein-losing gastroenteropathy]. Nihon Shokakibyo Gakkai zasshi = Jap. J. Gastroent. 105 (2008), 1775-1780.
56. Lim, KG, EC Rosenow, 3rd, B Staats, et al: Chyloptysis in adults: Presentation, recognition, and differential diagnosis. Chest 125 (2004), 336-340.
57. Maier, HC: Chylous reflux in the lungs and pleurae. Thorax 23 (1968), 281-296.
58. Taylor, JR, J Ryu, TV Colby, et al: Lymphangioleiomyomatosis. Clinical course in 32 patients. N. Engl. J. Med. 323 (1990), 1254-1260.
59. Chu, SC, K Horiba, J Usuki, et al: Comprehensive evaluation of 35 patients with lymphangioleiomyomatosis. Chest 115 (1999), 1041-1052.
60. Morimoto, N, S Hirasaki, T Kamei, et al: Pulmonary lymphangiomyomatosis (LAM) developing chylothorax. Int. Med. (Tokyo, Japan) 39 (2000), 738-741.
61. Frack, MD, L Simon, BH Dawson: The lymphangiomyomatosis syndrome. Cancer 22 (1968), 428-437.
62. van Lith, JM, HJ Hoekstra, WJ Boeve, et al: Lymphoedema of the legs as a result of lymphangiomyomatosis. A case report and review of the literature. J. Med. (Netherlands) 34 (1989), 310-316.
63. Abe, R, M Kimura, A Airosaki, et al: Retroperitioneal lymphangiomyomatosis with lymphedema of the legs. Lymphology 13 (1980), 62-67.
64. Radzikowska, E, P Jagus, A Skoczylas, et al: Role of serum vascular endothelial growth factor D in discrimination of patients with polycystic lung diseases. Polskie Archiwum Medycyny Wewnetrznej (2013), 533-538.
65. Young, LR, R Vandyke, PM Gulleman, et al: Serum vascular endothelial growth factor-D prospectively distinguishes lymphangioleiomyomatosis from other diseases. Chest 138 (2010), 674-681.
66. Young, LR, H-S Lee, Y Inoue, et al: Serum VEGF-D concentration as a biomarker of lymphangioleiomyomatosis severity and treatment response: A prospective analysis of the Multicenter International Lymphangioleiomyomatosis Efficacy of Sirolimus (MILES) trial. Lancet 1 (2013), 445-452.
67. Sengupta, S, TR Peterson, DM Sabatini: Regulation of the mTOR Complex 1 pathway by nutrients, growth factors, and stress. Molec. Cell 40 (2010), 310-322.
68. Taveira-DaSilva, AM, O Hathaway, M Stylianou, et al: Changes in lung function and chylous effusions in patients with lymphangioleiomyomatosis treated with sirolimus. Ann. Int. Med. 154 (2011), 797-805, w-292-793.
69. Makino, Y, Y Shimanuki, N Fujiwara, et al: Peritoneovenous shunting for intractable chylous ascites complicated with lymphangioleiomyomatosis. Int. Med. (Tokyo, Japan). 47 (2008), 281-285.
70. Laterre, PF, T Dugernier, MS Reynaert: Chylous ascites: Diagnosis, causes and treatment. Acta Gastro-enterologica Belgica 63(2000), 260-263.
71. Cárdenas, A, S Chopra: Chylous ascites. Am. J. Gastroent. 97 (2002), 1896-1900.
72. Hillerdal, G: Yellow nail syndrome: Treatment with octreotide. Clin. Resp. J. 1 (2007), 120-121.
73. Roehr, CC, A Jung, H Proquitte, et al: Somatostatin or octreotide as treatment options for chylothorax in young children: A systematic review. Inten. Care Med. 32 (2006), 650-657.
74. Demos, NJ, J Kozel, JE Scerbo: Somatostatin in the treatment of chylothorax. Chest 119 (2001), 964-966.
75. Kalomenidis, I: Octreotide and chylothorax. Curr. Opin. Pulmon. Med. 122 (2006), 264-267.
76. Min, HK, JI Jung, JS Song, et al: A 29-year-old woman with an intractable postoperative pleural effusion and pulmonary parenchymal opacification. Chest 142 (2012), 791-796.
77. Havas, E, T Parviainen, J Vuorela, et al: Lymph flow dynamics in exercising human skeletal muscle as detected by scintography. J. Physiol. 504 (1997), 233-239.

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INTERNAL MEDICINE

□ CASE REPORT □

An Intractable Case of Hermansky-Pudlak Syndrome

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Abstract

A 52-year-old Japanese man with congenital amblyopia and oculocutaneous albinism was admitted to our hospital. Chest CT showed reticular opacities and traction bronchiectasis without honeycombing. Specimens obtained by a video-assisted thoracoscopic surgery showed patchy chronic fibrotic lesions. We diagnosed him with Hermansky-Pudlak syndrome (HPS). A mutation in the *HPS1* gene was detected, and the diagnosis was confirmed. The patient was treated with prednisolone, pirfenidone, and azathioprine, but he nevertheless died within four months. Autopsy lung specimens showed diffuse alveolar damage suggesting comparatively rapid deterioration, although this presentation was not typical of an acute exacerbation. These pathological changes may be a possible progression pattern in HPS patients.

Key words: Hermansky-Pudlak syndrome, usual interstitial pneumonia, diffuse alveolar damage, pirfenidone

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Introduction

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive disease with the triad of albinism, a hemorrhagic tendency due to a platelet storage pool defect, and systemic accumulation of ceroid pigments (1). HPS consists of 9 genetically distinct subtypes, and patients with *HPS1*, *HPS2*, or *HPS4* mutations develop pulmonary fibrosis (2, 3). Among these subtypes, patients with the *HPS1* mutation have the highest incidence of pulmonary fibrosis (4), histologically characterized by usual interstitial pneumonia (UIP) (5). Treatment for pulmonary fibrosis caused by HPS with pirfenidone, an antifibrotic agent recently used to treat idiopathic pulmonary fibrosis (IPF), is controversial (6, 7). We herein describe a case of HPS that rapidly deteriorated despite treatment with prednisolone, pirfenidone, and azathioprine. We compared the pathological findings between surgical lung biopsy specimens and autopsy specimens to understand the mechanism of fatal pulmonary fibrosis in HPS. We detected diffuse alveolar damage (DAD), which was not observed in specimens from the video-assisted thoracoscopic surgery (VATS). These pathological changes may be an avenue to understand the mechanism of disease progression in HPS patients.

Case Report

A 52-year-old Japanese, non-smoking man who had congenital amblyopia and oculocutaneous albinism was referred to our hospital with an exacerbation of exertional dyspnea [grade 2 by the modified British Medical Research Council Scale (MRC)]. His brother and uncle had congenital albinism and died during childhood. In addition, his maternal grandfather was his paternal grandmother's sibling (Fig. 1). A physical examination revealed fine crackles at the basal lesions of both lungs. The patient's skin and hair were white and brown-colored, respectively. The laboratory data on admission revealed impaired clot retraction and platelet adhesiveness without thrombocytopenia or delayed coagulation time. The patient had increased serum levels of Krebs von den Lungen-6 (KL-6), surfactant protein (SP)-D and SP-A. A pulmonary function test revealed a restrictive pattern with impaired diffusion capacity (Table). During the six minute

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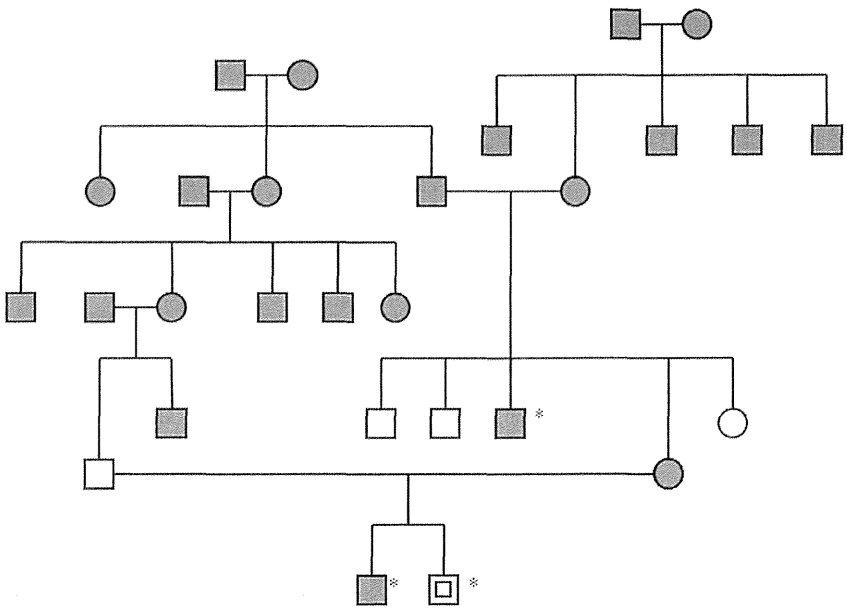

Figure 1. The pedigree of our HPS patient. The case (proband) is indicated by a doubled square. His maternal grandfather was his paternal grandmother's sibling. Asterisks (*) indicate relatives with oculocutaneous albinism. The patient died at 53 years of age, while his brother and his uncle died during childhood.

Table. Laboratory Data on Admission

Peripheral blood		
WBC	11,100	/μL
RBC	553×10^4	/μL
Hb	15.6	g/dL
Hct	38.7	%
Plt	39.7×10^4	/μL
ESR	53	mm/h
Blood chemistry		
LDH	367	IU/L
CRP	1.05	mg/dL
Glucose	185	mg/dL
HbA1c	10.0	%
Serology		
ANA	x80	
Homogeneous	x80	
Speckled	x80	
Other autoantibodies*	(-)	
KL-6	2,820	U/mL
SP-D	315	ng/mL
SP-A	210	ng/mL
Hemostasis and coagulation		
PT-INR	0.94	
APTT	24.2	seconds
Bleedind time	90	seconds
Fibrinogen	432.5	mg/dL
D-dimer	1.3	μg/mL
ATIII activity	110	%
Blood clot retraction	24.7	%
Platelet adhensiveness	21.5	%
ADP	48.0	%
Colloagen	45.0	%
PRP	36.1×10^4	/μL

Arterial blood gas analysis (room air)	
pH	7.418
PaO_2	91.5 Torr
$PaCO_2$	36.6 Torr
HCO_3^-	23.2 mEq/L
SaO_2	97.1 %
Pulmonary function test	
VC (%VC)	1.54 L (43.0%)
FVC (%FVC)	1.31 L (36.6%)
$FEV_{1.0}$ ($FEV_{1.0}$%)	1.31 L (100%)
RV (%RV)	0.90 L (45.5%)
RV/TLC	36.9 %
DLco (%DLco)	5.58 mL/min/mmHg (27.5%)
BALF analysis (rt. B^5b) 64/150 mL	
Total cell count	0.78×10^5/μL
Macrophage	76.0 %
Lymphocyte	3.4 %
Neutrophil	15.0 %
Eosinophil	5.6 %
CD4/8	1.30

* Other autoantibodies include antibodies for anti-DNA. Double-stranded DNA IgG, CCP, RNP, Jo-1, Scl-70, Sm, SS-A, SS-B, PR-ANCA and MPO-ANCA

walk test, the patient's oxygen levels fell below 85% at 95 seconds (103 meters). The chest X-ray showed bilateral diffuse reticular shadows. The high-resolution computed tomography (HRCT) showed reticular opacities and traction bronchiectasis mainly in the lower lung zone, but not honeycombing (Fig. 2). The lung specimens from the upper and

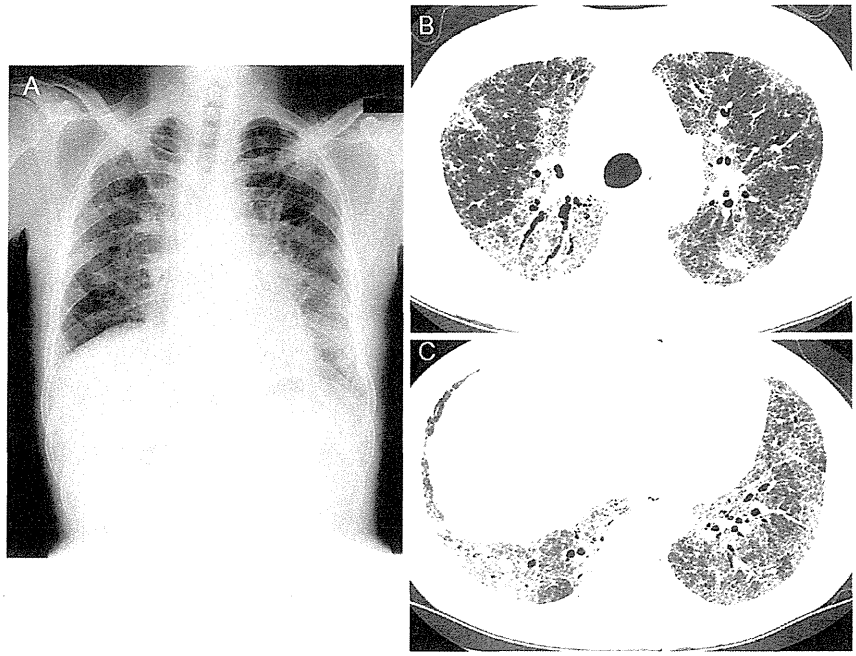

Figure 2. (A) The chest X-ray at the first visit to our hospital in February 2009 showed diffuse bilateral pulmonary infiltrates in all of the lung fields. (B, C) Computed tomography of the chest during the same visit showed bilateral reticular opacities predominantly in the subpleural and lower lung zones and traction bronchiectasis.

lower lobes of the left lung via VATS showed patchy chronic fibrotic lesions consistent with UIP. In the less fibrotic areas, there was a proliferation of pneumocytes with foamy cytoplasm (Fig. 3).

The patient demonstrated a progressive shortness of breath (grade 5 by the modified MRC at six weeks after VATS). We diagnosed him as having HPS and started treatment with prednisolone (30 mg daily). However, the patient's lung disease progressively deteriorated. We added pirfenidone to the treatment (initially 600 mg daily; thereafter increased to 1,800 mg daily). Simultaneously, azathioprine (50 mg daily) was administered to the patient. However, his respiratory failure progressed in proportion to the gradual deterioration of the bilateral diffuse reticular shadows on the chest X-ray, and he died four months after the start of treatment.

An autopsy was performed with permission from his family. Tissue sections of the skin did not contain melanin pigment in the epidermis. Ceroid pigments (brown pigments), negatively stained with the Berlin blue iron stain, were observed in the renal tubular epithelial cells and the bone marrow. The lung tissue from all lobes showed chronic fibrosing interstitial pneumonia (Fig. 4). We also observed alveolar lining cells with foamy cytoplasm in the less fibrotic regions (Fig. 4D), similarly observed in the biopsied lung tissues, characteristic of pulmonary fibrosis associated with HPS (5). Furthermore, we detected lesions of DAD and ring-like cystic lesions (Fig. 4C), which were formed in the process of DAD. A mutation of the *HPS1* gene, c.2003T>C, p.L668P homozygote or hemizygote, was detected after the patient expired, and the diagnosis of HPS was thus confirmed.

Discussion

Among Japanese HPS patients with the *HPS1* gene mutation, eight different types of genetic mutations have been reported (8). Functional analysis of the L668P variant, one of *HPS1* mutations, showed that this missense substitution is pathologic and leads to the inability of *HPS1* to assemble into a protein complex, namely the biogenesis of the lysosome-related organelles complex. As a result, it is supposed that cellular degeneration occurs with an overaccumulation of surfactant. In a recent report, the intracellular accumulation of surfactant was shown to lead to increased apoptosis of alveolar epithelial type II cells in a murine model of HPS, which represents a prominent reason for the development of lung fibrosis (2).

In HPS patients, pulmonary fibrosis, hemorrhage or colitis typically lead to death at an age of 40 to 50 years (9). In some patients with HPS, the interstitial lung disease worsens several years after the onset of pulmonary symptoms. To monitor disease status and progression, HRCT may be a useful tool (10, 11). The HRCT scores were shown to correlate with patient age and the extent of pulmonary dysfunction. Brantly et al. reported that the HRCT scores were also associated with the mortality of HPS patients (mean age: 37 years old); all patients with an HRCT score of 3 with severe disease (i.e., accompanying parenchymal consolidation, diffuse peribronchovascular thickening, traction bronchiectasis and reticulation) died of pulmonary fibrosis within four