

and it was assessed that it was not feasible to use mortality as the primary endpoint in the INPULSIS™ trials. Based on the 1-year survival rates observed in the TOMORROW study (89.2% of patients in the placebo group and 91.7% of patients in the nintedanib 150 mg twice daily group) it was calculated that a 1-year trial would require the inclusion of a total of approximately 6000 patients to provide 90% power to detect a difference between groups with a 2-sided *p*-value of 5%.

In the absence of an alternative explanation, a decrease in FVC in patients with IPF is consistent with progressive disease [1] and has been shown to be associated with reduced survival time in patients with IPF [26–32]. Change in FVC over 1 year has been used as a primary endpoint for Phase III clinical trials in patients with IPF [25,33]. The annual rate of decline in FVC – the primary endpoint in the INPULSIS™ trials – uses all the FVC values collected during the trial. This was considered to be a more robust methodology than using only the FVC value from baseline and 52 weeks because it enables calculation of the rate of decline even in patients without a week 52 value.

Several PROs for the assessment of the symptoms of IPF and the broader construct of HRQoL have been included as secondary endpoints in the INPULSIS™ trials. The SGRQ, chosen as a key secondary endpoint in the INPULSIS™ trials, has demonstrated acceptable psychometric characteristics in patients with IPF, including construct validity, reliability, and ability to detect change over time [22,34–36]. The two PROs used to assess dyspnoea, the UCSD-SOBQ and CASA-Q (CD), have been shown to have content validity in patients with IPF [37], with the UCSD-SOBQ also shown to detect change over time [36,38].

In the INPULSIS™ trials, acute exacerbations reported by the investigators will be assessed as a key secondary endpoint, as was done in the Phase II TOMORROW trial, in which a clinically relevant efficacy signal on acute exacerbations was observed. Furthermore, recent data suggest that suspected acute exacerbations (events that the investigator thinks are acute exacerbations but that cannot be adjudicated as acute exacerbations due to missing data or criteria) are clinically indistinguishable from confirmed acute exacerbations defined according to the consensus diagnostic criteria [39] and that both are clinically meaningful events [40]. Investigator-identified acute exacerbations were felt to best capture both definite and suspected acute exacerbations.

Conclusions

The INPULSIS™ trials will investigate the efficacy of nintedanib in patients with IPF, including its impact on disease progression as defined by decline in FVC, acute exacerbations and HRQoL. In addition, the data collected will characterise the adverse event profile of nintedanib in this patient population. The INPULSIS™ trials will report results in the first half of 2014. Together with the results of the other large ongoing randomized placebo-controlled trials in IPF, the INPULSIS™ trials will add significantly to scientific understanding of the natural history of IPF and will have potential implications for disease management.

Conflicts of interest

The INPULSIS™ trials were funded by Boehringer Ingelheim.

Luca Richeldi has received grants for research and fees for lectures, advisory boards meetings, and steering committee meetings from InterMune, Boehringer Ingelheim, Roche, Takeda, Shionogi, Biogen Idec, Sanofi-Aventis, MedImmune and ImmuneWorks.

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Yoshikazu Inoue has received fees for lectures, advisory board meetings, steering committee meetings, or safety boards from Boehringer Ingelheim, Shionogi, Bayer, Novartis Pharmaceuticals, Nobel Pharma, Teijin Pharma, Chugai Pharmaceutical, Takeda, Genzyme Japan and Eisai.

Martin Kolb has received consulting fees from Boehringer Ingelheim, InterMune and GlaxoSmithKline for activities related to IPF and his institution has received research grants from Boehringer Ingelheim, InterMune and GlaxoSmithKline.

Hiroyuki Taniguchi has received consultancy fees from Boehringer Ingelheim for steering committee meetings and advisory board meetings and from Olympus Corporation and AstraZeneca for advisory board meetings. He has received fees for lectures from Shionogi, Boehringer Ingelheim, Asahi Kasei Pharma Corp, Bayer, Chugai, GlaxoSmithKline, Ono Pharmaceutical Co, Teijin Pharma, AstraZeneca, Daiichi Sankyo, Eli Lilly, Novartis, Fukuda Denshi Co, Terumo Corp, Taiho Pharmaceutical Co, Kyorin Pharmaceutical Co, Meiji Seika Pharma Co, Philips Respironics, Pfizer, Abbott, Nippon Shinyaku Co, Eisai and Merck Sharp & Dohme.

David M. Hansell has received fees for consultancy and evaluating CT scans from Boehringer Ingelheim, InterMune and AstraZeneca.

Andrew G. Nicholson has no competing interests.

Ganesh Raghu has received travel reimbursements from Boehringer Ingelheim to attend scientific advisory board meetings.

Florence Le Maulf and Susanne Stowasser are employees of Boehringer Ingelheim.

Harold R. Collard has received consultancy fees (paid to his institution) from Biogen, FibroGen, Gilead, InterMune, Promedior, Pfizer, Bayer and Stromedix; grants from Boehringer Ingelheim, Genentech and the NIH/NHLBI; royalties (paid to his institution) from UpToDate; and payments for the development of educational presentations (paid to his institution) from Medscape.

Authors' contributions

All authors contributed to the study design and/or discussions regarding how the data from these trials would be analysed and interpreted. All authors contributed to the development of the manuscript. All authors have approved the final manuscript.

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Efficacy and Safety of Nintedanib in Idiopathic Pulmonary Fibrosis

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ABSTRACT

BACKGROUND

Nintedanib (formerly known as BIBF 1120) is an intracellular inhibitor that targets multiple tyrosine kinases. A phase 2 trial suggested that treatment with 150 mg of nintedanib twice daily reduced lung-function decline and acute exacerbations in patients with idiopathic pulmonary fibrosis.

METHODS

We conducted two replicate 52-week, randomized, double-blind, phase 3 trials (INPULSIS-1 and INPULSIS-2) to evaluate the efficacy and safety of 150 mg of nintedanib twice daily as compared with placebo in patients with idiopathic pulmonary fibrosis. The primary end point was the annual rate of decline in forced vital capacity (FVC). Key secondary end points were the time to the first acute exacerbation and the change from baseline in the total score on the St. George's Respiratory Questionnaire, both assessed over a 52-week period.

RESULTS

A total of 1066 patients were randomly assigned in a 3:2 ratio to receive nintedanib or placebo. The adjusted annual rate of change in FVC was -114.7 ml with nintedanib versus -239.9 ml with placebo (difference, 125.3 ml; 95% confidence interval [CI], 77.7 to 172.8 ; $P < 0.001$) in INPULSIS-1 and -113.6 ml with nintedanib versus -207.3 ml with placebo (difference, 93.7 ml; 95% CI, 44.8 to 142.7 ; $P < 0.001$) in INPULSIS-2. In INPULSIS-1, there was no significant difference between the nintedanib and placebo groups in the time to the first acute exacerbation (hazard ratio with nintedanib, 1.15 ; 95% CI, 0.54 to 2.42 ; $P = 0.67$); in INPULSIS-2, there was a significant benefit with nintedanib versus placebo (hazard ratio, 0.38 ; 95% CI, 0.19 to 0.77 ; $P = 0.005$). The most frequent adverse event in the nintedanib groups was diarrhea, with rates of 61.5% and 18.6% in the nintedanib and placebo groups, respectively, in INPULSIS-1 and 63.2% and 18.3% in the two groups, respectively, in INPULSIS-2.

CONCLUSIONS

In patients with idiopathic pulmonary fibrosis, nintedanib reduced the decline in FVC, which is consistent with a slowing of disease progression; nintedanib was frequently associated with diarrhea, which led to discontinuation of the study medication in less than 5% of patients. (Funded by Boehringer Ingelheim; INPULSIS-1 and INPULSIS-2 ClinicalTrials.gov numbers, NCT01335464 and NCT01335477.)

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*A complete list of investigators in the INPULSIS trials is provided in the Supplementary Appendix, available at NEJM.org.

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IDIOPATHIC PULMONARY FIBROSIS IS A FATAL lung disease characterized by worsening dyspnea and progressive loss of lung function.¹ A decline in forced vital capacity (FVC) is consistent with disease progression and is predictive of reduced survival time.¹⁻⁶

Idiopathic pulmonary fibrosis is believed to arise from an aberrant proliferation of fibrous tissue and tissue remodeling due to the abnormal function and signaling of alveolar epithelial cells and interstitial fibroblasts.⁷ The activation of cell-signaling pathways through tyrosine kinases such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and platelet-derived growth factor (PDGF) has been implicated in the pathogenesis of the disease.⁸⁻¹⁰

Nintedanib (formerly known as BIBF 1120) is an intracellular inhibitor that targets multiple tyrosine kinases, including the VEGF, FGF, and PDGF receptors.¹¹ The results of an earlier trial (To Improve Pulmonary Fibrosis with BIBF 1120 [TOMORROW]), a randomized, double-blind, placebo-controlled, phase 2 dose-finding study involving 432 patients with idiopathic pulmonary fibrosis, suggested that 12 months of treatment with 150 mg of nintedanib twice daily was associated with a reduced decline in FVC, fewer acute exacerbations, and the preservation of health-related quality of life.¹² We conducted two replicate phase 3 trials (INPULSIS-1 and INPULSIS-2) to evaluate the efficacy and safety of treatment with 150 mg of nintedanib twice daily in patients with idiopathic pulmonary fibrosis.

METHODS

STUDY DESIGN AND OVERSIGHT

The INPULSIS studies were randomized, double-blind, placebo-controlled, parallel-group trials performed at 205 sites in 24 countries in the Americas, Europe, Asia, and Australia. An independent data monitoring committee regularly reviewed the data, particularly serious adverse events, adverse events leading to discontinuation of the study drug, and the results of laboratory analyses, and made recommendations concerning the continuation of the trials. An adjudication committee that was independent of the investigators and whose members were unaware of the group assignments reviewed medical documentation to adjudicate the primary cause of all deaths. The committee also adjudicated all

adverse events reported by site investigators as acute exacerbations, in order to determine whether the events met the criteria for an acute exacerbation of idiopathic pulmonary fibrosis as defined in the protocol, available with the full text of this article at NEJM.org. The members of these committees are listed in Section B in the Supplementary Appendix, also available at NEJM.org.

Both trials were conducted in accordance with the principles of the Declaration of Helsinki and the Harmonized Tripartite Guideline for Good Clinical Practice from the International Conference on Harmonization and were approved by local authorities. The clinical protocol was approved by an independent ethics committee or institutional review board at each participating center. All patients provided written informed consent before study entry.

All the authors were involved in the design of the study and had access to the data, which were analyzed by the study sponsor, Boehringer Ingelheim. All the authors vouch for the accuracy and completeness of the data analyses and the fidelity of each study to the protocol. The protocol and statistical analysis plans are available at NEJM.org. The manuscript was drafted by the first, second, and last authors and revised by all the authors. Medical writing assistance, paid for by Boehringer Ingelheim, was provided by the Fleishman-Hillard Group.

PATIENTS

Patients were eligible to participate in the two trials if they were 40 years of age or older and had received a diagnosis of idiopathic pulmonary fibrosis within the previous 5 years. Additional eligibility criteria were an FVC that was 50% or more of the predicted value, a diffusion capacity of the lung for carbon monoxide (DLCO) that was 30 to 79% of the predicted value, and high-resolution computed tomography (HRCT) of the chest performed within the previous 12 months. HRCT images (for all patients) and lung-biopsy specimens (if available) were reviewed centrally by a single radiologist and a single pathologist to verify eligibility according to the protocol. Eligibility criteria with regard to findings on HRCT and surgical lung biopsy are shown in Table S1 and Table S2, respectively, in the Supplementary Appendix.

Concomitant therapy with up to 15 mg of prednisone per day, or the equivalent, was permitted if the dose had been stable for 8 or more

weeks before screening; patients receiving other therapies for idiopathic pulmonary fibrosis, including high-dose prednisone, azathioprine, N-acetylcysteine, and any investigational treatments for idiopathic pulmonary fibrosis, were excluded. After 6 months of study treatment, patients whose condition had deteriorated could receive azathioprine, cyclophosphamide, cyclosporine, N-acetylcysteine, or more than 15 mg of prednisone per day, or the equivalent, at the discretion of the investigator. In cases of acute exacerbation reported by an investigator at any time during the trial, any treatments could be initiated or doses increased as deemed appropriate by the investigator. Other key exclusion criteria are listed in Section C in the Supplementary Appendix.

STUDY PROTOCOL

After a screening period, eligible patients were randomly assigned in a 3:2 ratio to receive 150 mg of nintedanib twice daily or placebo for 52 weeks. An interactive telephone and Web-based response system was used to perform randomization. Patients, investigators, and the study sponsor were unaware of the study-group assignments throughout the study. Completion of the 52-week treatment period was followed by a follow-up visit 4 weeks later. Spirometric tests were conducted at baseline; at 2, 4, 6, 12, 24, 36, and 52 weeks; and at the follow-up visit. Spirometric testing was conducted in accordance with criteria published by the American Thoracic Society and the European Respiratory Society.¹³ All spirometric measurements were performed on machines provided by the sponsor, and the results were centrally reviewed, with training and ongoing feedback provided for the site investigators.

Dose interruption or reduction of the dose from 150 mg twice daily to 100 mg twice daily was allowed for the management of adverse events. After an adverse event had resolved, the dose could be reinstated at 150 mg twice daily. The site investigators were provided with recommendations for the management of diarrhea and elevated levels of liver enzymes. To minimize the amount of missing data, patients who discontinued the study drug prematurely were asked to attend all scheduled visits and to undergo all examinations as originally planned. For patients who discontinued the drug prematurely but did not agree to attend all visits, data on vital status were collected at week 52.

END POINTS

The primary end point for both INPULSIS trials was the annual rate of decline in FVC (measured in milliliters per year). Key secondary end points were the time to the first acute exacerbation (as reported by a site investigator) and the change from baseline in the total score on the St. George's Respiratory Questionnaire (SGRQ), both assessed over the 52-week treatment period. The SGRQ is a self-administered questionnaire that is used to assess health-related quality of life. It comprises three domains (symptoms, activity, and impact). The total score and the score for each domain range from 0 to 100, with higher scores indicating worse health-related quality of life.^{14,15} A minimally important difference in the score has not been established for patients with idiopathic pulmonary fibrosis; in patients with chronic obstructive pulmonary disease, this difference is 4 points.¹⁶ Patients completed the SGRQ at baseline and at 6, 12, 24, and 52 weeks. Acute exacerbations were defined as events meeting all of the following criteria: unexplained worsening or development of dyspnea within the previous 30 days; new diffuse pulmonary infiltrates visualized on chest radiography, HRCT, or both, or the development of parenchymal abnormalities with no pneumothorax or pleural effusion (new ground-glass opacities) since the preceding visit; and exclusion of any known causes of acute worsening, including infection, left heart failure, pulmonary embolism, and any identifiable cause of acute lung injury, in accordance with routine clinical practice and microbiologic studies. All acute exacerbations reported by the site investigators were categorized by the adjudication committee, whose members were unaware of the study-group assignments, as confirmed or suspected or were not considered to be an acute exacerbation according to prespecified criteria.¹⁷

Other prespecified secondary end points included the absolute change from baseline in FVC (in milliliters and as a percentage of the predicted value) over the 52-week treatment period, the proportion of patients with an FVC response (defined as the proportion of patients in whom the percentage of predicted FVC did not decline by more than 5 percentage points or by more than 10 percentage points at week 52), the risk of an acute exacerbation, the change from baseline in SGRQ domain scores over the 52-week treatment period, death from any cause, death

from a respiratory cause, and death that occurred between randomization and 28 days after the last dose of the study drug. All mortality end points were measured as the time to death. Safety was assessed by means of clinical and laboratory evaluation at study visits and the recording of adverse events.

STATISTICAL ANALYSIS

Efficacy and safety analyses were conducted for patients who were randomly assigned to a study group and received at least one dose of the study medication. The primary end point was analyzed with the use of a random coefficient regression model (with random slopes and intercepts) that included sex, age, and height as covariates. The treatment effect was determined by using estimated slopes for each study group (on the basis of the time-by-treatment interaction term from the mixed model). All available FVC values from baseline to week 52 were used in the primary model, including FVC measurements at the follow-up visit for patients who discontinued the study medication prematurely and did not complete the study visits through week 52. The statistical model used for the primary analysis allowed for missing data, assuming that they were missing at random; missing data were not imputed for the primary analysis, but data collected after discontinuation of the study drug were used in the primary analysis. Significance tests were two-sided, with an alpha value of 0.05.

The superiority of nintedanib over placebo with respect to the primary and key secondary end points was tested with the use of a hierarchical procedure to account for multiple comparisons (see Section D in the Supplementary Appendix). Sensitivity analyses were performed to assess the robustness of the results for the primary and key secondary end points. Multiple imputation sensitivity analyses were performed to assess the effect of missing data and provide estimates of the treatment effect under different assumptions about missing data (Fig. S2 in the Supplementary Appendix). For the time to the first acute exacerbation, a sensitivity analysis based on the occurrence of confirmed or suspected acute exacerbations (as determined by the adjudication committee) in pooled data from the two trials was prespecified.

The frequency and severity of adverse events were documented according to the *Medical Diction-*

ary for Regulatory Activities, version 16.1. Safety analyses were descriptive. For information on the statistical analysis of other end points, see Section D in the Supplementary Appendix.

For each trial, the sample size was calculated to provide 90% power to detect a between-group difference of 100 ml in the annual rate of FVC decline. On the basis of data from the phase 2 trial, the standard deviation for the change in FVC from baseline was assumed to be 300 ml in both groups. Assuming that it would not be possible to evaluate data for 2% of patients, the sample size was calculated as 194 patients in the placebo group and 291 patients in the nintedanib group for a two-group t-test at a one-sided significance level of 2.5%. Since the primary analysis was based on a random coefficient regression model that included adjustment for several variables and took into account information across time, we expected that the power would be greater than the 90% calculated for the t-test.

RESULTS

PATIENTS

Between May 2011 and September 2012, a total of 1066 patients underwent randomization: 515 patients in INPULSIS-1 and 551 patients in INPULSIS-2 (Fig. S1 in the Supplementary Appendix). In INPULSIS-1, a total of 513 patients received at least one dose of the study medication (309 received nintedanib and 204 received placebo). A total of 78 patients (25.2%) in the nintedanib group and 36 patients (17.6%) in the placebo group discontinued the study medication prematurely. Of these patients, 31 (39.7%) in the nintedanib group and 11 (30.6%) in the placebo group completed visits up to week 52. The most frequent reason for premature discontinuation of the study medication was at least one adverse event (65 patients [21.0%] in the nintedanib group and 24 [11.8%] in the placebo group). In INPULSIS-2, a total of 548 patients received at least one dose of the study medication (329 received nintedanib and 219 received placebo). A total of 78 patients (23.7%) in the nintedanib group and 44 patients (20.1%) in the placebo group discontinued the study medication prematurely. Of these patients, 26 (33.3%) in the nintedanib group and 10 (22.7%) in the placebo group completed visits up to week 52. The most frequent reason for premature discontinu-

ation of the study medication was at least one adverse event (62 patients [18.8%] in the nintedanib group and 35 [16.0%] in the placebo group). The proportion of patients with missing FVC data at week 52 was approximately 15%; the proportion of patients with missing data did not differ significantly between the nintedanib and placebo groups (Fig. S2 in the Supplementary Appendix).

In each trial, the baseline characteristics of patients in the nintedanib and placebo groups were similar (Table 1, and Table S3 in the Supplementary Appendix). The mean duration of exposure to the study drug in the nintedanib and placebo groups was similar (approximately 45 weeks in each trial), but a higher proportion of patients in the nintedanib group than in the placebo group had dose reductions or interrup-

Table 1. Baseline Characteristics of Patients in INPULSIS-1 and INPULSIS-2.*

Characteristic	INPULSIS-1		INPULSIS-2	
	Nintedanib (N=309)	Placebo (N=204)	Nintedanib (N=329)	Placebo (N=219)
Male sex — no. (%)	251 (81.2)	163 (79.9)	256 (77.8)	171 (78.1)
Age — yr	66.9±8.4	66.9±8.2	66.4±7.9	67.1±7.5
Weight — kg	82.0±16.8	81.2±16.3	76.6±15.9	76.3±16.5
Body-mass index†	28.6±4.5	28.1±4.6	27.6±4.6	27.2±4.5
Smoking status — no. (%)				
Never smoked	71 (23.0)	51 (25.0)	103 (31.3)	71 (32.4)
Former smoker	217 (70.2)	144 (70.6)	218 (66.3)	139 (63.5)
Current smoker	21 (6.8)	9 (4.4)	8 (2.4)	9 (4.1)
Time since diagnosis of idiopathic pulmonary fibrosis — yr	1.7±1.4	1.6±1.4	1.6±1.3	1.6±1.3
Specimen from surgical lung biopsy available — no. (%)	60 (19.4)	33 (16.2)	84 (25.5)	52 (23.7)
Systemic corticosteroid therapy — no. (%)‡	68 (22.0)	43 (21.1)	68 (20.7)	46 (21.0)
FVC				
Mean — ml	2757±735	2845±820	2673±776	2619±787
Median — ml	2700	2721	2615	2591
Percentage of predicted value	79.5±17.0	80.5±17.3	80.0±18.1	78.1±19.0
FEV ₁ :FVC (%)	81.5±5.4	80.8±6.1	81.8±6.3	82.4±5.7
DlCO				
mmol/min/kPa	4.0±1.2	4.0±1.1	3.8±1.2	3.7±1.3
Percentage of predicted value§	47.8±12.3	47.5±11.7	47.0±14.5	46.4±14.8
Spo ₂ — %	95.9±2.0	95.9±1.9	95.8±2.6	95.7±2.1
Total SGRQ score¶	39.6±17.6	39.8±18.5	39.5±20.5	39.4±18.7

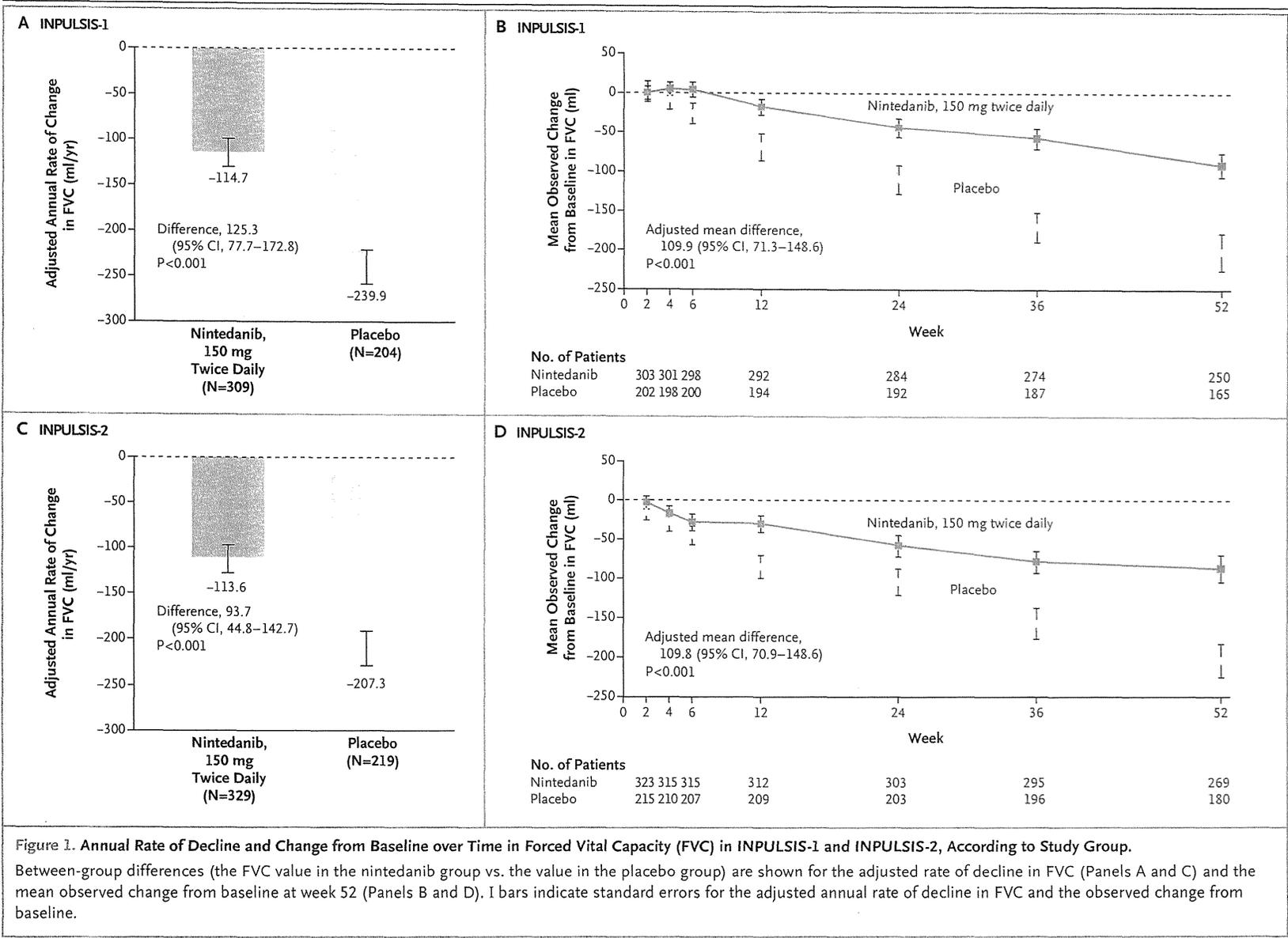
* Plus-minus values are means ±SD. FEV₁ denotes forced expiratory volume in 1 second, FVC forced vital capacity, and Spo₂ oxygen saturation of peripheral blood.

† The body-mass index is the weight in kilograms divided by the square of the height in meters.

‡ Prednisone at a dose of no more than 15 mg per day or the equivalent was permitted if the dose had been stable for at least 8 weeks before screening.

§ The percentage of the predicted value for the diffusion capacity of the lung for carbon monoxide (DlCO) was calculated with the use of the equation described by the European Community for Steel and Coal in Cotes et al.¹⁸ In INPULSIS-2, data were available for 218 patients in the placebo group.

¶ In INPULSIS-1, the total score on the St. George's Respiratory Questionnaire (SGRQ) was available for 298 patients in the nintedanib group and 202 patients in the placebo group; in INPULSIS-2, the total SGRQ score was available for 326 patients in the nintedanib group and 217 patients in the placebo group. The total score ranges from 0 to 100, with higher scores indicating worse health-related quality of life.



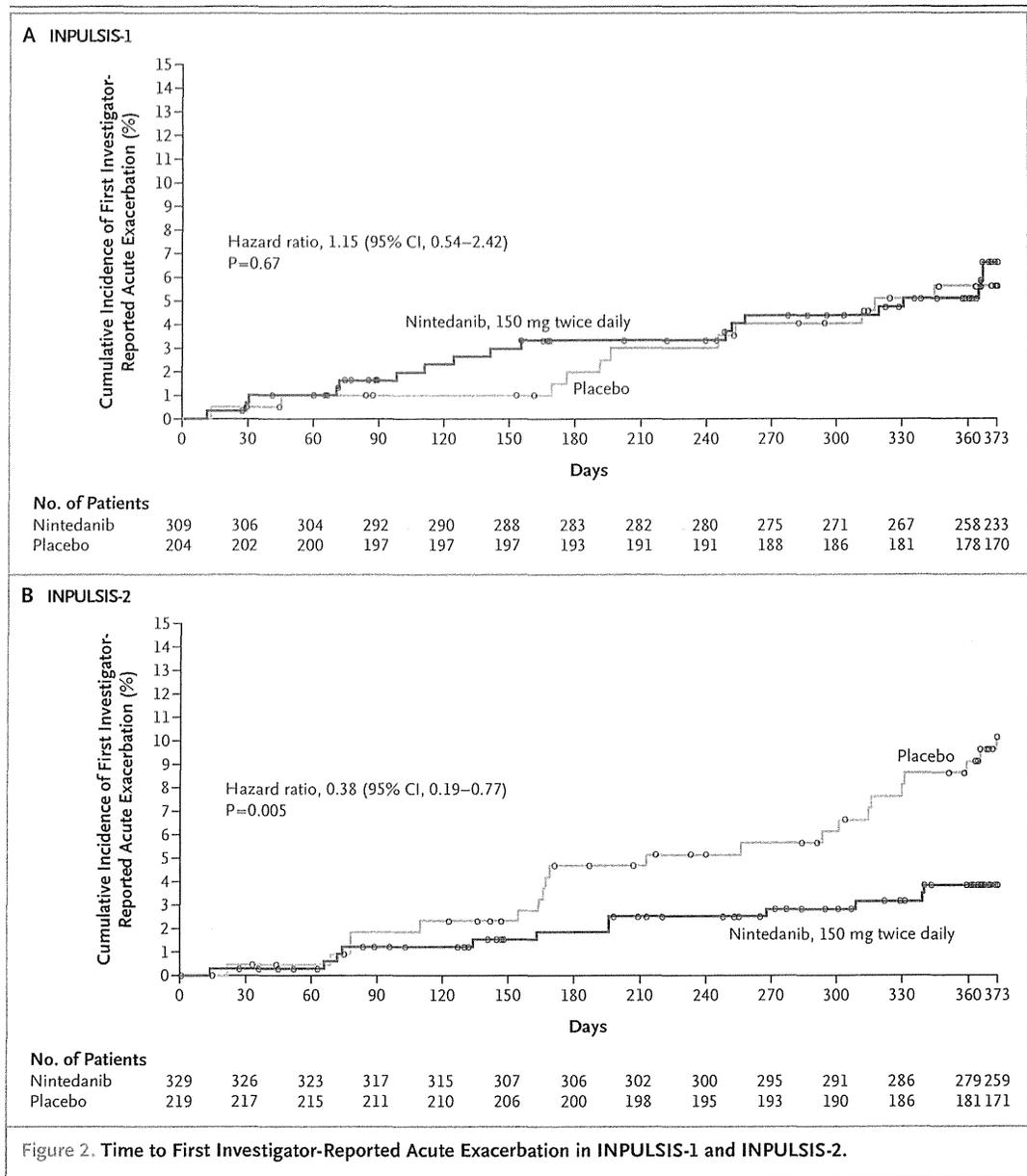


Figure 2. Time to First Investigator-Reported Acute Exacerbation in INPULSIS-1 and INPULSIS-2.

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 propor-

tion 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)
	<i>number of patients (percent)</i>			
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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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, chylocolporrheal 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%, lymphangiomyomas 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 to 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,

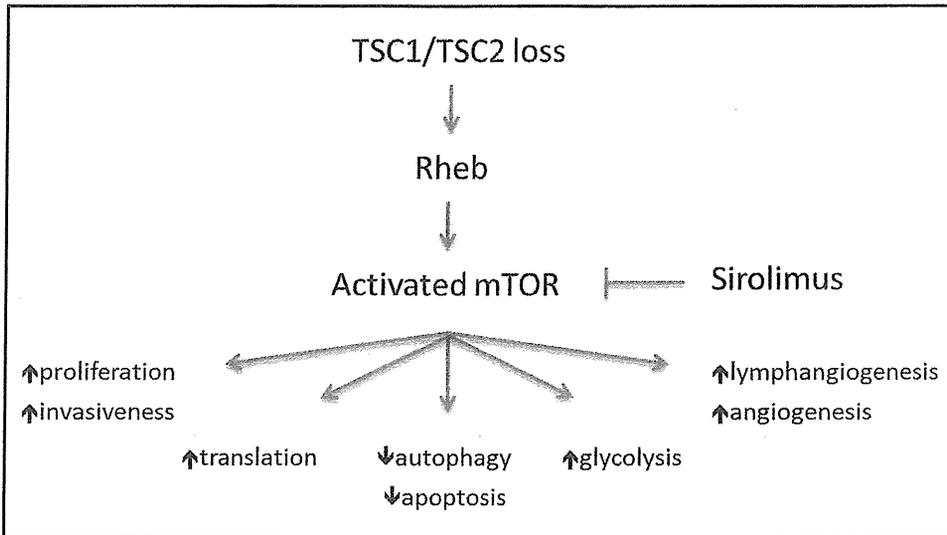


Fig. 1. Signaling networks in LAM cells. TSC1 and TSC2 form a complex that integrates input from upstream signaling cascades, such as those emanating from membrane tyrosine kinase receptors. Rheb, which is normally suppressed by TSC1/TSC2, becomes activated when TSC1 or TSC2 are defective or deficient.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

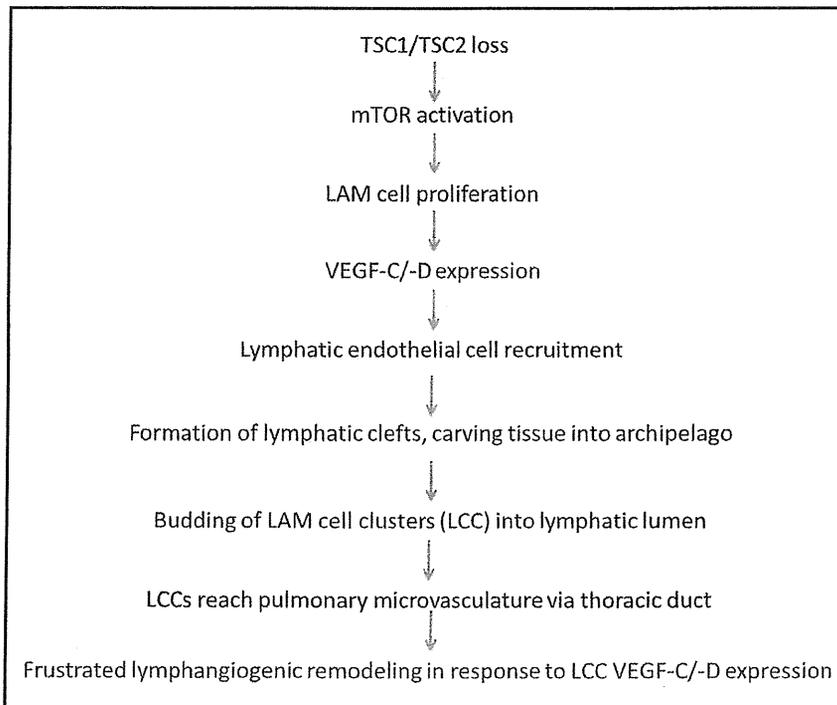


Fig. 2. Role of TSC mutations and lymphatic processes in the pathogenesis of LAM.

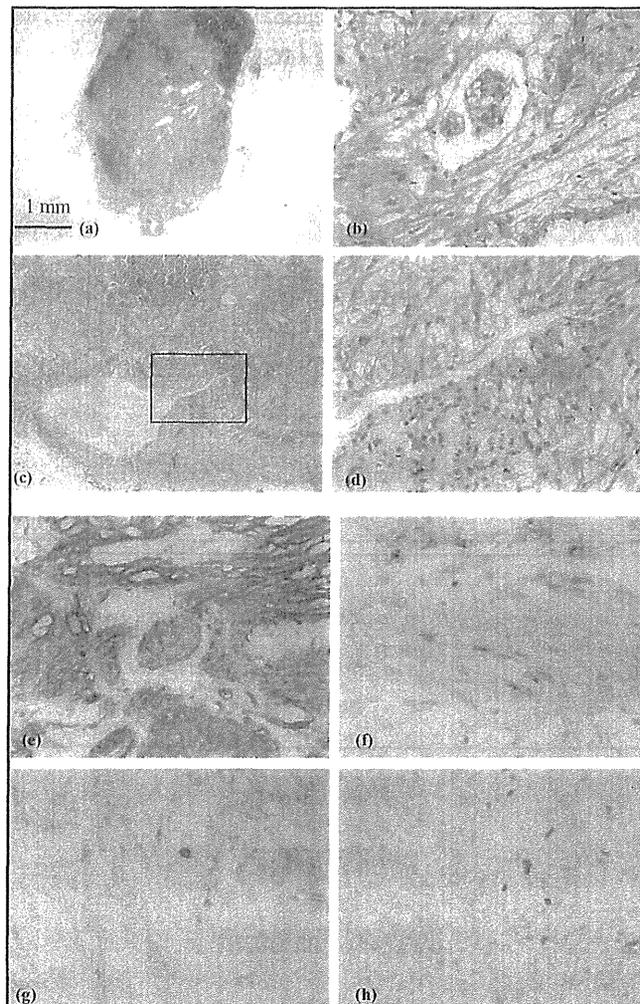


Fig. 3. (a-h) LAM histology and immunohistochemical staining. a) Involvement of a lymph node by LAM. About 70% of this lymph node was replaced by bundles of immature smooth muscle cells (H&E stain, x1). Higher magnifications (a) revealed two LAM cell clusters in a lymphatic vessel within the lymph node (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. Lymphangioliomyomas 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).