ERJ Express. Published on October 31, 2013 as doi: 10.1183/09031936.00141013

Serum Lysyl Oxidase Like-2 Levels and Idiopathic Pulmonary Fibrosis Disease Progression

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Authors' contribution: All authors 1) substantially contributed to conception and design, acquisition of data, or analysis and interpretation of data; 2) substantially contributed to drafting the article or revising it critically for important intellectual content; and 3) final approval of the version to be published.
Funding Source: Gilead Sciences Inc and NIH grant P50HL084932.

Running Title: sLOXL2 and risk for IPF disease progression

Descriptor Number: 9.23 Interstitial Lung Disease

Total Manuscript Word Count: 3,431

Abstract Word Count: 200

ABSTRACT

We evaluated whether lysyl oxidase-like 2 (LOXL2), which promotes cross-linking of collagen in pathologic stroma, was detectable in serum from idiopathic pulmonary fibrosis (IPF) patients, and assessed its relationship with IPF disease progression (DP).

Patients from ARTEMIS-IPF (n=69) and the Genomic and Proteomic Analysis of Disease Progression in IPF (GAP) study (n=104) were analyzed. Baseline serum LOXL2 levels were compared to baseline clinical and physiologic surrogates of disease severity, and the association with IPF DP was assessed using a classification and regression tree (CART) method.

sLOXL2 correlated weakly with forced vital capacity and carbon monoxide diffusion capacity (r range -0.24 to 0.05) in both cohorts. CART-determined thresholds were similar: ARTEMIS-IPF 800pg/mL, GAP 700pg/mL. In ARTEMIS-IPF, higher sLOXL2 (>800pg/mL) was associated with increased risk for DP (hazard ratio [HR] 5.41, 95% confidence interval [CI] 1.65-17.73). Among GAP subjects with baseline spirometric data (n=70), higher sLOXL2 levels (>700pg/mL) were associated with more DP events (HR 1.78, 95% CI 1.01-3.11). Among all GAP subjects, higher sLOXL2 levels were associated with increased risk for mortality (HR 2.28, 95% CI 1.18-4.38).

These results suggest that higher sLOXL2 levels are associated with increased risk for IPF DP. However, due to multiple limitations, these results require validation.

Summary of Important Findings

Higher baseline sLOXL2 levels doesn't correlate with IPF severity, but is associated with higher risk for disease progression.

INTRODUCTION

Idiopathic pulmonary fibrosis (IPF) is a progressive and ultimately fatal disease of the lungs involving airway epithelial cell damage, fibroblast activation and proliferation, and excessive deposition of collagen and other extracellular matrix (ECM) components [1]. Increased deposition of fibrillar collagens I and III is apparent [2], along with changes in the abundance and localization of collagens V, XII, and XIV, which help to organize and stabilize the growing fibrils [3, 4]. These modifications of ECM composition and organization alter the biomechanical properties of the lung parenchyma, and increase local tension, which is critical in IPF disease pathogenesis [5].

One important driver of matrix tension is lysyl oxidase-like 2 (LOXL2), an enzyme that catalyzes covalent crosslinking of ECM molecules, including fibrillar collagens. Activated fibroblasts secrete LOXL2 along with increased amounts of collagen [6]. This pathologic remodeling of the ECM likely results in the release of latent TGFβ from the latency-associated peptide (LAP) complex sequestered in the ECM [7, 8], driving a feed-forward loop of cell activation, LOXL2 and collagen secretion, and matrix stiffening.

LOXL2 protein expression is observed in the fibroblastic foci and collagenous regions of diseased IPF lung tissue, with relatively minor expression in healthy lung tissue [9]. LOXL2 has also been localized to the active disease interface in liver fibrosis [9], and is considered a "core" driver in fibrosis [10]. Since LOXL2 is associated with areas of active fibrogenesis in diseased tissues, we hypothesized that serum levels of LOXL2

(sLOXL2) would be elevated in IPF patients. We developed an immunoassay capable of relative quantification of the circulating levels of LOXL2 protein, and tested serum samples collected from two independent cohorts of well-characterized IPF patients. The main objective of this study was to evaluate whether sLOXL2 levels at baseline are associated with measures of IPF disease severity and the risk for IPF progression. Preliminary results of this study have been previously reported at several international meetings [11, 12].

METHODS

This study was conducted using serum and clinical data collected from subjects participating in two independent clinical studies: ARTEMIS-IPF and the Genomic and Proteomic Analysis of Disease Progression (GAP) in Idiopathic Pulmonary Fibrosis Study. All serum and data were collected prospectively, but analyzed retrospectively. All analyses were approved by the institutional review boards of each clinical site.

Study populations and serum collection

ARTEMIS-IPF (ClinicalTrials.gov identifier NCT00768300) was a multi-center, randomized, double-blind, placebo-controlled, event-driven trial that evaluated whether ambrisentan, a selective antagonist of the ET_A receptor approved for treatment of pulmonary arterial hypertension, could prevent IPF disease progression [13]. Baseline clinical and physiologic data included percent of predicted forced vital capacity (FVC), percent of predicted carbon monoxide diffusion capacity (DL_{CO}), 6-minute walk distance

(6MWD), mean pulmonary artery pressure (PAP), the St. George's Respiratory Questionnaire (SGRQ) score and the Transition Dyspnea Index (TDI) score. The PAP was obtained via a right heart catheterization, which was required of all study subjects at baseline. The composite physiologic index (CPI) was calculated using the baseline FVC and DL_{CO} according to a previously published formula [14].

The GAP study (ClinicalTrials.gov identifier NCT00373841) is a prospective observational study that recruits subjects at the University of Pittsburgh Dorothy P. and Richard P. Simmons Center for Interstitial Lung Disease. Recruitment for this cohort began in October 2005. The database was locked for the current analysis on December 1, 2011. The study subjects were required to be 1) ≥ 18 years of age 2) have a diagnosis of IPF according to ATS/ERS guidelines [15] and 3) able to receive all ongoing care and follow-up at the clinical facility. Clinical data collected between three months prior or two weeks after study visit 1 were included as part of the baseline assessment, which included but was not limited to lung function testing.

For ARTEMIS-IPF, baseline serum was collected prior to randomization at select clinical sites in the United States. These sites had the necessary infrastructure and support staff for collection of these samples as specified according to the laboratory protocol. For the GAP cohort, baseline serum samples were collected at enrollment study visit, then every 4-6 months thereafter during routine clinic visits. After collection, serum samples were aliquoted and frozen at -80°C; all serum samples analyzed for this study were maintained at -80°C until testing.

Healthy donor serum

Serum LOXL2 levels were analyzed in samples from self-reported "healthy" adult donors purchased from Bioreclamation Inc. (Hicksville, NY) and Cureline Inc. (South San Francisco, CA). Donors were seronegative for hepatitis B, hepatitis C, and HIV infection.

Clinical endpoints

The same pre-specified clinical endpoints used in the ARTEMIS-IPF clinical trial were used for the sLOXL2 analysis. The primary endpoint was time to the first IPF disease progression event, a composite endpoint composed of mortality from any cause, adjudicated respiratory hospitalizations, or a categorical decrease in lung function (defined as either a ≥10% decrease in FVC with a ≥5% decrease in the DL_{CO} or a ≥15 % decrease in DL_{CO} with a ≥5% decrease in FVC). For the GAP cohort, the ARTEMIS-IPF disease progression definition was applied to the subset of subjects with baseline spirometric measurements (n=70), but without including respiratory hospitalization events. Hospitalizations were not tracked in the GAP cohort because these patients were referred from other healthcare programs, where they were hospitalized when necessary. All-cause mortality was evaluated among all subjects with baseline sLOXL2 available for analysis (n=104). If lung transplantation occurred during the follow-up period, it was considered a mortality event.

Antibody generation and sLOXL2 immunoassay

Details of the antibody generation methods and sLOXL2 immunoassay procedure and validation can be found in the online supplement. Briefly, two anti-LOXL2 antibodies, one rabbit polyclonal and one mouse monoclonal, were generated against full-length purified LOXL2 protein. A sandwich immunoassay using an electrochemiluminescent platform (Meso Scale Discovery, Rockville, MD) was developed using these two anti-LOXL2 antibodies. Purified recombinant human LOXL2 protein (R&D Systems, Minneapolis, MN) was used as the calibrator, allowing for relative quantification of unknown samples.

Statistical analysis

A staged approach was used to analyze the ARTEMIS-IPF and GAP cohorts. The first stage consisted of 9 association analyses in the ARTEMIS-IPF cohort, 8 focused on baseline characteristics and 1 focused on the disease progression endpoint. A Bonferroni correction for multiple comparisons required the alpha-threshold set at 0.006. The second stage was a hypothesis driven analysis of the GAP cohort, where the primary analysis evaluated whether the association between baseline sLOXL2 levels with IPF disease progression can be replicated at the alpha threshold of 0.05. All testing with the components of the disease progression endpoint was considered exploratory, given each of these endpoints was a component of the disease progression endpoint.

All analyses were performed using SAS v.9.1. Patient samples with sLOXL2 levels below the assay lower limit of detection ([LLOD] 180 pg/mL) were assigned the value LLOD/2 (90 pg/mL) for purposes of analysis, as recommended for left-censored data

[16]. Pearson's correlation coefficient was used to determine correlations between sLOXL2 levels and demographic and clinical variables. A classification and regression trees (CART) method, with IPF disease progression status (binary) as response variable and baseline sLOXL2 level as dependent variable, was used to select the "low" versus "high" sLOXL2 subjects to evaluate the association between the IPF disease progression and baseline sLOXL2. CART was applied in an unsupervised fashion independently to each cohort. Differences in probability of developing an endpoint for high and low sLOXL2 groups were calculated using cumulative incidence curves. The association between an endpoint and sLOXL2 levels was assessed independently for each cohort using Cox proportional hazard modeling. A stepwise selection method was used to identify baseline variables that were associated with the endpoint of interest at the $p<0.05$ threshold. Treatment assignment was forced into the models for the ARTEMIS-IPF analyses. Follow-up for ARTEMIS-IPF was for the full duration of the study. GAP cohort subjects were followed until death, lung transplant or censored at 24- months after enrollment. Statistical significance was defined as a 2-sided p-value of <0.05.

RESULTS

LOXL2 Immunoassay and Characterization of Healthy Donors

Development of the LOXL2 immunoassay was conducted using calibrator samples generated by adding recombinant full-length human LOXL2 protein into serum from normal healthy donors lacking detectable LOXL2 levels as described in the supplemental Methods section. Incurred patient samples were used for validation. Assay performance

characteristics for serum samples are shown in Table 1; assay performance for EDTA plasma samples was similar (data not shown).

Samples from self-reported healthy adult donors (n=101) unrelated to the IPF study participants were tested for sLOXL2 levels. The mean age of these donors (± standard deviation) was 50.3 ± 14.3 and 60% were males; There was no association between age (p=0.36) with sLOXL2 levels. 90% (91 donors) had sLOXL2 levels <LLOD and 2% (2 donors) had sLOXL2 levels <LLOQ.

LOXL2 in the ARTEMIS-IPF Study

Among the 492 subjects in the intent-to-treat cohort, 69 (14%) subjects (49 treated with ambrisentan) had baseline serum samples collected and available for analysis. The mean follow-up time for these subjects was 245 days. There were 9 deaths during this follow-up period for this subgroup with serum available. These subjects, in comparison to subjects without baseline serum, had similar baseline demographic characteristics and IPF severity, as reflected by % of predicted FVC and DL_{CO}, the 6MWD, mean PAP, CPI, SGRQ score, and the TDI score (Table 2).

The median baseline sLOXL2 level for all 69 subjects was 304 pg/mL (Figure 1; interquartile range [IQR] 144, 738; minimum <180 pg/mL [below LLOD], maximum 5,389 pg/mL); the median baseline sLOXL2 level for placebo- and ambrisentan-treated subjects was 206.1 pg/mL and 360.6 pg/mL respectively. Correlation was weak between sLOXL2 and baseline demographic and physiologic measures of IPF severity (correlation

coefficients: age 0.31, FVC -0.24, DL_{CO} -0.13, 6MWD -0.04, PAP 0.17, CPI 0.14, SGRQ 0.12, TDI -0.18). CART analysis of baseline sLOXL2 levels identified 800 pg/mL as the optimal threshold for classifying the IPF disease progression risk in this cohort; 36 (67%) and 18 (33%) ambrisentan treated subjects were subgrouped as having "low" (≤800 pg/mL) versus "high" (>800 pg/mL) baseline sLOXL2 levels respectively; 13 (87%) and 2 (13%) placebo treated subjects were subgrouped as having "low" (≤800 pg/mL) versus "high" (>800 pg/mL) baseline sLOXL2 levels respectively.

Presence of a high baseline sLOXL2 level was associated with higher probability for experiencing IPF disease progression events (Figure 2A). This association was consistently observed for all three components of the disease progression endpoint: lung function decline (Figure 2B), respiratory hospitalizations (Figure 2C), and mortality (Figure 2D). Cox proportional hazard modeling (Table 3) revealed that a high baseline sLOXL2 level was associated with a 5.4-fold increase in risk for IPF disease progression (p=0.005), a 7.6-fold increase in risk for lung function decline (p=0.031), and a 5.4-fold increase in risk for respiratory hospitalizations (p=0.029). Treatment assignment was included in each of these models, as were 6MWD and CPI scores, which were the only baseline variables found to remain significant using a stepwise approach.

sLOXL2 in the GAP Cohort

Overall, 120 subjects were enrolled into the GAP cohort. Among these, 105 had serum samples available for analysis, of which 1 subject did not have adequate clinical data. Of the remaining 104 subjects, 70 had baseline spirometry values. In comparison to

ARTEMIS-IPF, GAP subjects tended to have lower baseline FVC but slightly higher DL_{CO} (Table 2). The mean follow-up time was also longer at 455 days. All data were censored at 2 years after enrollment for the remaining analyses. During this period, there were 26 deaths and 17 lung transplants in this cohort.

The median baseline sLOXL2 level for the 104 subjects was 716 pg/mL (Figure 1; IQR <180 pg/mL [below LLOD], 1447; minimum <180 pg/mL [below LLOD], maximum 15,708). In the subset of subjects with baseline spirometry data available, correlation between the baseline sLOXL2 levels and lung function was weak (correlation coefficients: age -0.16, FVC -0.01, DL_{CO} -0.28, CPI 0.24). CART analysis identified 700 pg/mL as the optimal threshold for classifying the cohort for IPF disease progression risk; 46 subjects (44%) were subgrouped as having "low" (≤700 pg/mL) sLOXL2, 58 subjects (56%) were subgrouped as having "high" (>700 pg/mL) sLOXL2.

Among the 70 subjects with baseline spirometric measurements, presence of a high baseline sLOXL2 level was associated with significantly more IPF disease progression events (p=0.042, Figure 3A). Cox proportional hazard model (Table 3) revealed that high baseline sLOXL2 level was associated with a 1.8-fold increase in risk for disease progression (p=0.045). Although lung function decline (p=0.334) and mortality (p=0.153), when considered as individual components, did not meet the 0.05 p-value threshold, presence of a high baseline sLOXL2 level was associated with a higher probability of experiencing one of these events (Figure 3B and 3C). Mortality analysis of all 104 subjects in the GAP cohort revealed that high baseline sLOXL2 levels were

associated with more deaths (p=0.017, Figure 3D), and a 2.2-fold increase in risk for death (p=0.020; Table 4). Each of these statistical models included sex and age as covariates, which were the only baseline variables found to remain significant using a stepwise approach.

Discussion

LOXL2 is directly involved in disease-related fibrogenesis [9]: it promotes cross-linking of fibrillar collagen I, a major component of desmoplastic stroma and fibrosis, and plays a crucial role in matrix remodeling, fibrogenesis, and formation of pathologic stroma. In this study, we demonstrated that LOXL2 can be detected in circulation, and among patients with IPF, we found a novel association between sLOXL2 levels and IPF disease progression. We also found that baseline sLOXL2 levels were not significantly correlated with typical clinical measures of IPF disease severity (e.g. FVC and DL_{CO}). Given the current understanding of LOXL2's role in fibrosis, these findings suggest that circulating sLOXL2 levels may be reflective of IPF disease activity, rather than severity.

Although these results represent an association and not a direct causal relationship between sLOXL2 levels and IPF outcomes, there are several reasons to believe that sLOXL2 is worthy of further investigation as a potential biomarker for IPF disease activity. First, there is strong biologic evidence for a causal relationship between high sLOXL2 levels and poor IPF outcomes. We demonstrated previously that LOXL2 is expressed in murine models of fibrotic disease (e.g. lung fibrosis and liver fibrosis), and can be inhibited by treatment with an anti-LOXL2 antibody [9]. In a therapeutic murine

model of pulmonary fibrosis, GS-607601 (formerly known as AB0023), a mouse monoclonal antibody that inhibits LOXL2, was able to reduce LOXL2 levels in the lung, reduce factors that reflect fibrotic activity (e.g. number of αSMA-positive fibroblasts, SDFα-1, TGFβ-1, endothelin-1, pSMAD2) and reduce the extent of fibrosis. In humans with hepatitis B- or C-associated liver fibrosis, high sLOXL2 levels have also been demonstrated to be associated with higher Ishak fibrosis score, and sLOXL2 levels decline with anti-hepatitis B therapy [17, 18]. Thus, both nonhuman and human data support the possibility that sLOXL2 is reflective of the extent of fibrotic disease activity.

Second, sLOXL2 was consistently associated with disease progression and its components in both IPF cohorts. In ARTEMIS-IPF, the LF decline and RH components of the disease progression endpoint were most strongly associated with high sLOXL2 levels. The association with mortality risk also trended in the same direction, although it did not meet the 0.05 p-value threshold. In the GAP cohort, both the LF decline and mortality risks met the 0.05 p-value threshold. Significantly, the mortality risk associated with high sLOXL2 levels in the GAP cohort was of similar magnitude to that observed in ARTEMIS-IPF, suggesting that if the mortality event rate were higher in ARTEMIS-IPF, the 0.05 p-value threshold would have likely been achieved.

Third, these associations were observed in two independent populations that are quite different on multiple levels, setting our analysis up as a sensitivity analysis for the relationship between baseline sLOXL2 and IPF clinical outcomes. The ARTEMIS-IPF population was recruited within the context of a well-controlled multicenter clinical trial in which the inclusion and exclusion criteria were designed to identify subjects with mild IPF; subjects with >5% honeycombing on HRCT were excluded [19]. The GAP cohort is an observational cohort referred for evaluation at an interstitial lung disease program located in a tertiary institution where patients are more likely to have advanced IPF. Although the FVC and DL_{CO} were similar between these two cohorts, the number of mortality events was quite different between these 2 cohorts (Table 2).

Despite the biologic plausibility and statistical significance of this association between sLOXL2 levels and IPF outcomes, there remain significant limitations that should be considered. These observations were made using small IPF cohorts, which may result in imprecise point estimates. Although treatment assignment was forced into the Cox models, the risk estimates associated with sLOXL2 in the ARTEMIS-IPF analysis also remains subject to residual confounding effects of the study drug, which was associated with a 1.7 fold increase in risk for IPF disease progression [19]. Exclusion of 30% of the GAP subjects due to missing baseline lung function data may have also biased the results. Subjects with missing baseline lung function data may have had more advanced IPF disease, as reflected by more deaths (50% versus 37%) and a slightly higher risk for death (HR 1.75, 95% CI 0.95-3.23, p=0.07). Finally, the low picogram levels of sLOXL2 require an assay of extremely high sensitivity. We expect that the CART identified

sLOXL2 thresholds may change when a more robust and standardized assay is developed and a more comprehensive IPF population has been surveyed.

Given the high likelihood that LOXL2 is involved in the pathogenesis of IPF, future studies should examine the diagnostic, prognostic and predictive potential of sLOXL2. It is possible that sLOXL2 may enhance the positive predictive value of current diagnostic criteria [15] for IPF. Future studies should also consider whether sLOXL2, along with other promising prognostic IPF biomarkers (e.g. MMP1, MMP7, KL-6, periostin, surfactant protein-A and D, CCL-18, VEGF, YKL-40) [20-30], as well as prognostic scores [31, 32] and radiologic modalities [33], might have prognostic value for helping physicians and patients anticipate the patient's IPF disease progression. This might include evaluation of serially collected sLOXL2 levels and their relationship to IPF acute exacerbations, which represent a terminal event for many IPF patients [34]. Finally, sLOXL2 levels might be predictive of an IPF patient's response to targeted therapy. Simtuzumab, a humanized monoclonal antibody that is an allosteric inhibitor of LOXL2, is being investigated in a Phase 2 clinical trial (ClinicalTrials.gov identifier NCT01769196) for treatment of IPF. This study will help to determine whether baseline sLOXL2 levels predict response to simtuzumab, as well as confirm our findings presented in this paper that patients with high baseline LOXL2 levels are at increased risk for poor IPF outcomes.

In summary, these data demonstrate a novel association between higher sLOXL2 levels and increased risk for poor IPF outcomes. Given this association was demonstrated in

two independent IPF populations and that LOXL2 has been shown to be a disease-driver of pathogenesis of fibrotic diseases in animal models, this association may be causal in nature. However, these results do not indicate that sLOXL2 is a suitable biomarker for IPF at this time. Future work should evaluate the epidemiology of sLOXL2 in not only patients with IPF, but all fibrotic lung diseases, which will be necessary to determine whether sLOXL2 has any diagnostic, prognostic, or predictive value in the management of IPF patients.

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Table 1. sLOXL2 immunoassay performance characteristics.

Assay characteristic	Result
Accuracy (relative error)	< 15%
Intra-assay precision (coefficient of variation)	5%
Inter-assay precision (coefficient of variation)	25%
Lower limit of detection (LLOD)	180 pg/mL
Lower limit of quantitation (LLOQ)	440 pg/mL
Upper limit of quantitation (ULOQ)	Absolute limit not determined; highest calibrator standard used was 132,118 pg/mL

Accuracy was determined using pooled serum (from healthy individuals) spiked with recombinant LOXL2 protein.
Intra-assay and inter-assay precision were determined using incurred samples.
LLOD = the mean + 2.5*SD of the blank wells.
LLOQ = the lowest calibrator standard with relative error < 30% and coefficient of variation < 30%.

Table 2. Baseline characteristics and measurements of subjects in the ARTEMIS-IPF study and GAP cohort.

Baseline Characteristics and Measurements	ARTEMIS-IPF		GAP Cohort (n=104)
	No Serum (n=423)	Serum (n=69)	
Mean Age (SD)	65.9 (7.3)	66.2 (7.0)	66.7 (8.9)
Number of Males	303 (72%)	52 (75%)	73 (70%)
Mean % Predicted FVC (SD)	69.0% (13.5)	69.8% (12.1)	66.1% (17.7) †
Mean % Predicted DL_{CO} (SD)	43.4% (14.1)	42.1% (11.1)	47.8% (18.0) †
Mean 6MWD (SD)	416.2m (120.1)	398.6m (115.8)	NA
Mean PAP mmHg (SD)	20.4 (7.1)	20.4 (5.9)	NA
Mean CPI Score (SD)	52.2 (10.8)	52.5 (8.9)	51.0 (13.0)
Mean SGRQ Score (SD)	39.4 (19.5)	38.0 (18.4)	NA
Mean TDI Score (SD)	7.3 (2.4)	7.5 (2.4)	NA
Median days of follow-up (SD)	237 (143)	245 (177)	455 (340)
Number of deaths	23 (5%)	9 (13%)	43 (41%)*

†Only 70 (67%) subjects had baseline pulmonary function data available.
*Censored at 24 months after enrollment, lung transplant (n=17) was treated as a mortality event
SD=standard deviation; FVC=forced vital capacity; DLCO=carbon monoxide diffusion capacity; 6MWD=6-minute walk distance; PAP=pulmonary artery pressure; CPI=composite physiologic index; SGRQ=Saint George's Respiratory Questionnaire; TDI=transition dyspnea index; NA=not applicable

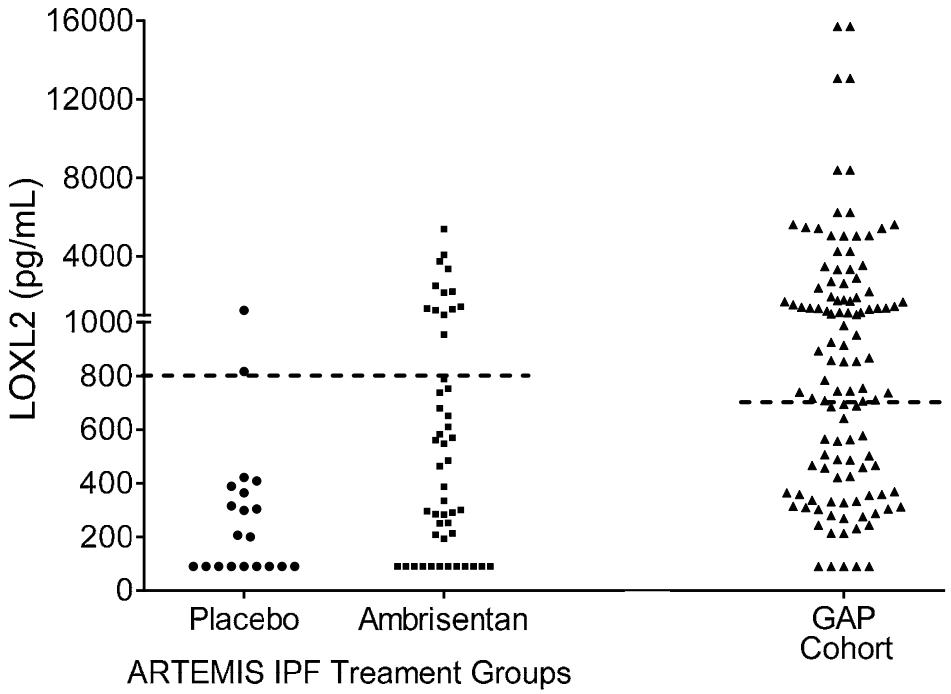
Table 3. Hazard ratios for endpoints in the ARTEMIS-IPF study according to low versus high baseline sLOXL2.

Endpoints	Event Rate		Hazard ratio (95% confidence interval) for high sLOXL2	P-value
	Low sLOXL2	High sLOXL2		
ARTEMIS-IPF†				
Disease progression	10/54 (19%)	8/15 (53%)	5.41 (1.65-17.73)	0.005
Lung function decline	5/54 (9%)	4/15 (27%)	7.64 (1.21-48.25)	0.031
Respiratory hospitalization	6/54 (11%)	6/15 (40%)	5.35 (1.19-24.00)	0.029
Mortality	5/54 (9%)	4/15 (27%)	1.87 (0.28-12.45)	0.517
GAP*				
Disease progression	22/35 (63%)	28/35 (80%)	1.78 (1.01-3.11)	0.045
Lung function decline	13/35 (37%)	16/35 (46%)	1.43 (0.69-2.98)	0.337
Mortality	10/35 (29%)	16/35 (46%)	1.77 (0.80-3.89)	0.159
Mortality, all subjects	13/46 (28%)	30/58 (52%)	2.28 (1.18-4.38)	0.014

† Each model for ARTEMIS-IPF included treatment assignment, 6MWD, and CPI as covariates.

*The first 3 models (disease progression, lung function decline and all-cause mortality) included only subjects with baseline spirometry (n=70). These models included no covariates. The second mortality model that included all subjects (n=104) included sex as a covariate.

Figure 1. Distribution of sLOXL2 among subjects treated with ambrisentan and placebo in ARTEMIS-IPF and subjects in the GAP cohort. The dotted lines represent thresholds used for dichotomization of subjects in to "low" and "high" sLOXL2 groups in ARTEMIS-IPF (800pg/mL) and GAP cohort (700pg/mL).



Figures 2A-D. Cumulative incidence curves comparing low (≤800 pg/mL) and high (>800 pg/ml) sLOXL2 levels for disease progression (A) and its components (lung function decline [B], respiratory hospitalizations [C] and death [D]) in ARTEMIS-IPF.

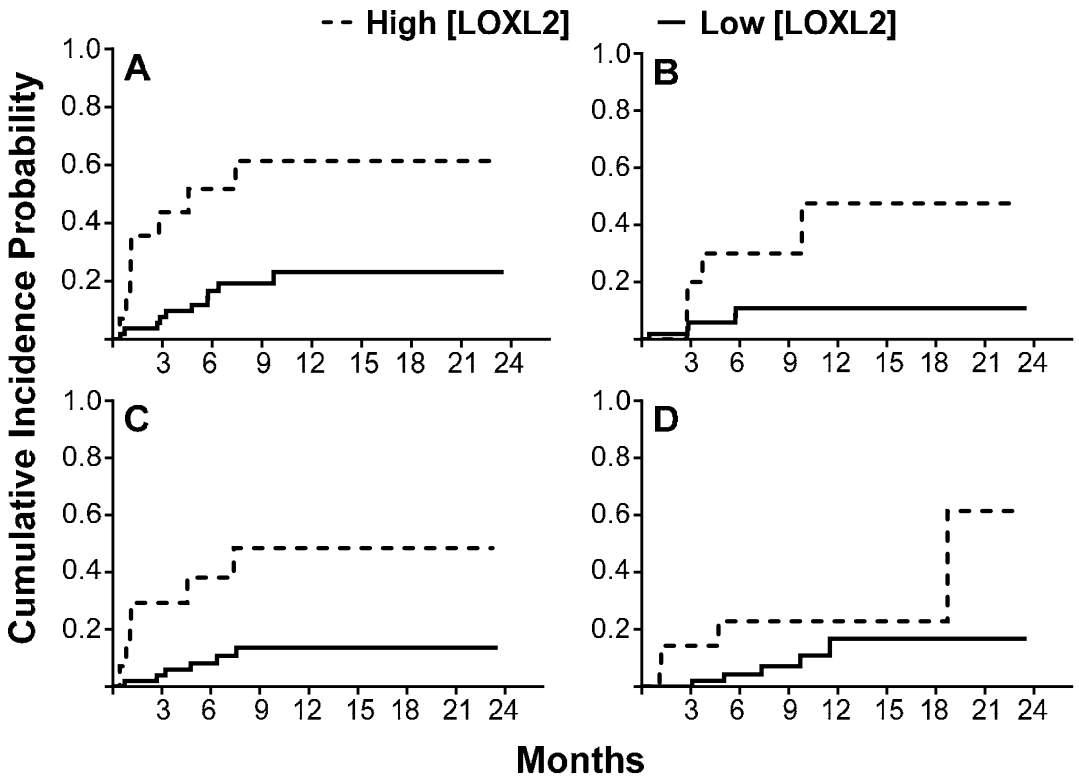


Figure 3A-D. Cumulative incidence curves comparing low (≤700 pg/mL) and high (>700 pg/ml) sLOXL2 levels for disease progression (A) and its components (lung function decline [B] and death [C and D]) among GAP subjects with (n=70 [C]) and without (n=104, [D]) baseline spirometric values.