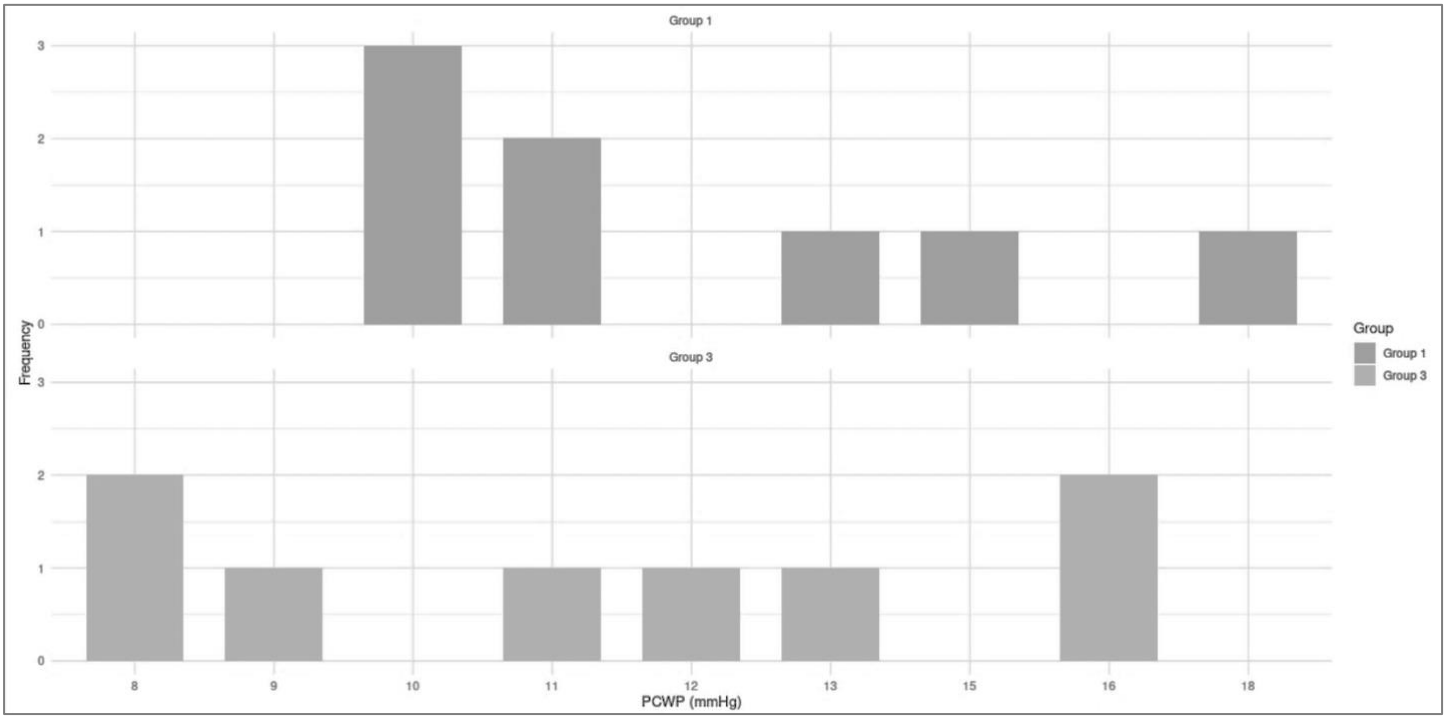
SUPPLEMENTAL



Supplemental Figure 1. Distribution of PCWP at screening by PH group. Bar plot showing frequencies of PCWP values at time of participant screening to determine study inclusion. These frequencies are displayed by PH group. PCWP = pulmonary capillary wedge pressure (mmHg). There were three participants with PCWP measurements greater than 15 mmHg at time of screening. One participant with group 1 PAH had a PCWP of 18 mmHg and two participants with group 3 PH-ILD had a PCWP of 16 mmHg. To avoid inclusion of patients with group 2 PH, we excluded patients with evidence of left heart disease, valvular disease, and more than three risk factors for left ventricular heart disease.

SUPPLEMENTAL

Supplemental Table 1. Trial enrollment enriched for patients with history of autoimmune disease

Autoimmune Medical History at Baseline, listed by alphabetical order
• Autoimmune hepatitis • CREST syndrome • Hashimoto's disease • Myositis • Primary biliary cirrhosis • Psoriatic arthritis • Raynaud's phenomenon • Rheumatoid arthritis • Scleroderma • Systemic lupus erythematosus • Systemic sclerosis

SUPPLEMENTAL

Supplemental Table 2. List of inclusion and exclusion criteria.

Inclusion Criteria	• Adults ages 18 to 75 years inclusive • WHO functional class II/III/IV despite treatment with maximally tolerated doses of 2 or more treatment modalities including PDE5 inhibitors, guanylate cyclase stimulators, endothelin receptor antagonists, and prostanoids when appropriate. • Baseline 6MWT>100 meters and <550 meters • Established diagnosis of Group 3 pulmonary hypertension secondary to interstitial lung disease OR established diagnosis of Group 1 pulmonary hypertension secondary to connective tissue disease, idiopathic, hereditary, drugs, or toxins. • Right heart catheterization at randomization showing precapillary pulmonary hypertension (mPAP ≥ 25 mmHg and PVR > 400 dynes * sec * cm -5) and: PCWP ≤20 mmHg for Group 3 PH patients and Group 1 PAH patients • Participants on chronic medication for PAH, PH, or underlying lung disease must be on a stable and optimized dose for at least 90 days prior to the first dose of the study drug. • Female participants who are heterosexually active must use an acceptable method of contraception: condoms (male or female) with or without a spermicidal agent, diaphragm or cervical cap with spermicide, IUD, or Hormone-based contraceptive • Be able to provide written informed consent and comply with requirements of the study
Exclusion Criteria	• Participants with a diagnosis of PAH or PH for reasons other than the pulmonary hypertension primarily due to any of the following: o Group 2, 4, or 5 o Group 1 due to HIV, veno-occlusive disease, porto-pulmonary hypertension, congenital heart disease o Group 3 due to severe chronic obstructive pulmonary disease (COPD) or obstructive sleep apnea (OSA) o Note: participants with overlapping syndromes will be evaluated on a case-by-case basis by the recruiting physician • Total Lung Capacity (TLC) <60% predicted • FEV1/FVC <50% predicted or FEV1 <55% predicted • Inability to safely attempt completion of the 6MWD test • Use of experimental PAH treatments within the past 3 months • Current systemic treatment with hymecromone • Left sided heart disease as defined by either a PCWP >20 mmHg and/or left ventricular ejection fraction <40% o Note: participants with abnormal left ventricular function attributable entirely to impaired left ventricular filling due to the effects of right ventricular overload (i.e. right ventricular hypertrophy and/or dilatation) are not excluded • Participants must not have 3 or more of the following left ventricular disease / dysfunction risk factors: o Body mass index (BMI) > 30kg/m^2 o History of essential hypertension requiring medication o Diabetes mellitus o Historical evidence of significant coronary disease established by any of the following: - History of myocardial infarction - History of percutaneous coronary intervention or coronary artery bypass graft

	- Angiographic evidence of >50% stenosis in at least one coronary artery - Positive stress test with imaging - Stable angina • Significant valvular heart disease as determined by more than moderate findings on echocardiogram or history of valve replacement • Pregnant or actively breastfeeding • Female participants with childbearing potential not willing to use a form of birth control (including abstinence) during the study • Inability to undergo right heart catheterization • Acute pulmonary embolism within 90 days of randomization • Exacerbation of underlying lung disease or active pulmonary or upper respiratory infection within 30 days of randomizations • Use of any inhaled tobacco products or significant history of drug abuse within 3 months prior to randomization • Subject is receiving >10L/min of oxygen supplementation by any mode of delivery at rest at baseline • Body mass index > 40kg/m^2 • Participants with history of dysphagia, achalasia, or difficulty swallowing capsules, tablets or pills • Participants with liver failure or AST or ALT greater than 2 times the upper limit of normal • Participants with total bilirubin levels greater than 2 times the upper limit of normal • Participants with CrCl <45 • Use of any investigational drug/device, or participation in any investigational study with therapeutic intent within 30 days prior to randomization • • Known allergy to hymecromone or any component of drug product ((including rare hereditary problems of galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption) • Known allergy to any component of placebo (including rare hereditary problems of galactose intolerance, Lapp lactase deficiency, or glucose-galactose malabsorption) • Physician concern that participant may not adhere to the study protocol • Significant psychiatric, addictive, or other disorder that compromises the subject's ability to provide informed consent

SUPPLEMENTAL

Supplemental Table 3. Comprehensive list of study endpoints.

Primary Endpoint	• Change in pulmonary vascular resistance (PVR) measured by right heart catheterization (RHC) from baseline to end of treatment (Week 24)
Secondary Endpoints	• The safety and tolerability of H01 in adults with pulmonary hypertension using the Common Terminology Criteria for Adverse Events (CTCAE) • Change in mean pulmonary arterial pressure (mPAP) by RHC from baseline to end of treatment (Week 24) • 6 Minute Walk Distance Test from baseline to end of treatment (Week 24) • Change in quality of life (QOL) score, EmPHasis-10 score and St George Respiratory Questionnaire (SGRQ) score from baseline to end of treatment (Week 24) • Change in serum HA concentration from baseline to end of treatment (Week 24) • Change in NT-proBNP from baseline to end of treatment (Week 24)
Exploratory Endpoints	• Change in inflammatory markers and PH-specific biomarkers (ESR, CRP, D-dimer, WCC, ferritin, LDH, NLRC3) • Change in pro-inflammatory cytokines • Change in Forced Expiratory Volume in one second (FEV1) • Change in Forced Vital Capacity (FVC) from pulmonary function test (PFT) • Change in Total Lung Capacity (TLC) from pulmonary function test (PFT) • Change in Lung diffusion capacity (DLCO) from pulmonary function test (PFT) • Change in exhaled breath condensate (EBC) hyaluronan concentrations over the study period • Describe the pharmacokinetics (H01 and metabolite serum concentrations) • Describe HA fragment size

SUPPLEMENTAL

Supplemental Table 4. Protocol for 6-minute walk test (6MWT).

The 6MWT for SATURN will be conducted within established American Thoracic Society guidelines and standards [24].

Briefly, supervised walks will be conducted in a proper hallway with a minimum of 100-foot distance where participants can conduct the test uninterrupted.
The 6MWT for this protocol will be performed at Screening, Week 4, Week 12, and Week 24. The data gathered during these walk tests include walk distance, walk duration, number of laps walked, vitals and Borg ratings at pre, peak, and post walk, and utilization of walk aids and supplemental oxygen. The following instructions will be utilized for walk tests for all subjects and at all assessments to ensure continuity and standardization.

The 6MWT is to be performed at the same pre-approved walking course at all visits, which is 30 meters in length. The walking course is marked at start and finish of each lap, as well as at 5-meter intervals. Emergency personnel and equipment should be accessible nearby in the event of an emergency. The same 6MWT equipment (measuring tape, stopwatch, lap counter, cones) should be utilized at all walk tests. The walking course should have cones placed to indicate turnaround points. Subjects will be reminded to wear the same comfortable shoes at each assessment. They will also be informed not to exercise within two hours of the assessment. Subjects must rest at least 10 minutes prior to the assessment.

6MWT administrators will be required to ensure the following:

- The walk assessment should only be performed after laboratory samples or ECG for the applicable visit are obtained. Performance of the walk tests before these assessments are prohibited as this can result erroneous laboratory and ECG results.
- Walking aids (i.e. walker, cane, crutches etc.) must be the same across all visits from Screening to Week 24. If no walking aid is used at Screening but is required for use at subsequent visits due to safety concerns, this will be permitted and documented by walk administrator. All attempts will be made to ensure continuity, but this can be amended at the discretion of the investigator or walk administrator in interest of patient safety.
- Supplemental oxygen use must be the same across all visits from Screening to Week 24. This includes flow rate and mode of administration across all visits at and at pre, peak, and post walk at all walk tests. The novel administration of oxygen or increased flow rate will be permitted in circumstances where there are safety concerns. These changes will be clearly documented, and can occur pre, peak, or post walk. All attempts will be made to ensure continuity, but this can be amended at the discretion of the investigator or walk administrator in interest of patient safety.
- If a face mask is worn at the Screening walk test, a face mask of the same type will also be worn at subsequent walk tests.

The six-minute walk test should not be performed or stopped if the subject experiences the following symptoms (including, but not limited to): severe dyspnea, dizziness, resting heart rate >120, blood pressure > 180/100, chest pain, leg cramps, staggering, or excessive diaphoresis. These symptoms will be documented.

The same, pre-approved Borg Scale will be used to assess Borg ratings at pre, peak, and post walk for all subjects at all visits. The same vitals machine will be used to assess vitals for patients at pre, peak, and post walk at each assessment.

If the patient stops walking during the 6MWT, rest times and rest duration will be documented. If the patient does not resume walking or if the administrator terminates the assessment, stop time and duration will be recorded, and the rationale for stopping the walk will be provided.

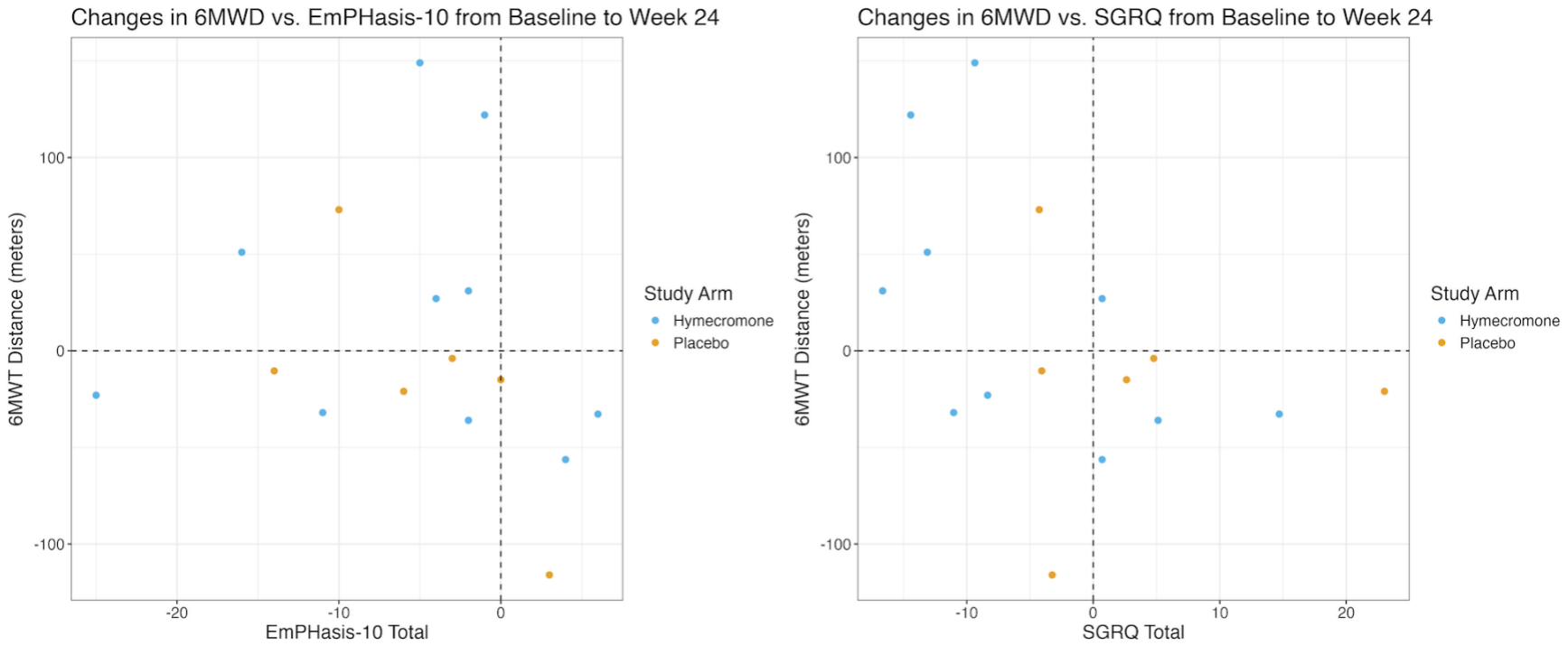
If the 6MWT cannot be performed (acute injury, subject refusal, worsening symptoms, etc.), the investigator will be notified, and subsequent action should be taken at the discretion of the investigator. This should be documented.

A repeat 6MWT may be performed at the discretion of the investigator.

All efforts will be made to ensure the same 6MWT administration performs the 6MWTs for all subjects.

Walk distances are not to be disclosed to subjects under any circumstances.

SUPPLEMENTAL



Supplemental Figure 2. Changes in 6MWT distance versus EmPHasis-10 and SGRQ from baseline to week 24, respectively. Scatter plot showing change in individual 6-minute walk distance (m) versus change in total EmPHasis-10 and SGRQ, respectively. 6MWD = 6-minute walk distance test, EmPHasis-10 = Evaluating Measurement of Pulmonary Hypertension Symptoms-10 Questionnaire, SGRQ = St. George's respiratory questionnaire.

SUPPLEMENTAL

Supplemental Table 5. Post-hoc analyses of the primary outcome, PVR

	Estimated mean difference (95% CI)[a]	p-value[a]	Effect size (95% CI)[b]	p-value[c]
PVR, Wood units	0.6 (-1.5, 2.7)	0.54	-0.2 (-0.9, 0.8)	0.51

[a] calculated using cLDA

[b] calculated using Glass rank biserial correlation coefficient

[c] calculated using Wilcoxon Rank Sum test

SUPPLEMENTAL

Supplemental Table 6. Summary of Pulmonary Function Test results (Group 1 PAH), reported as mean (SD).

	Hymecromone (N=5)		Placebo (N=3)	
Timepoint	**Baseline**	**Week 24**	**Baseline**	**Week 24**
% Predicted FEV_1	93.6 (16.5)	94.4 (13.1)	74.7 (7.5)	73.0 (9.0)
% Predicted FVC	94.4 (16.7)	96.2 (14.0)	76.3 (11.9)	76.3 (13.6)
% Predicted TLC	94.2 (18.0)	98.0 (13.3)	90.0 (5.6)	92.0 (6.6)
% Predicted DLCO, mL/min/mmHg	71.8 (39.8)	71.2 (44.5)	87.0 (4.0)	87.3 (2.9)

FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, TLC = total lung capacity, DLCO = diffusing capacity of the lung for carbon monoxide.

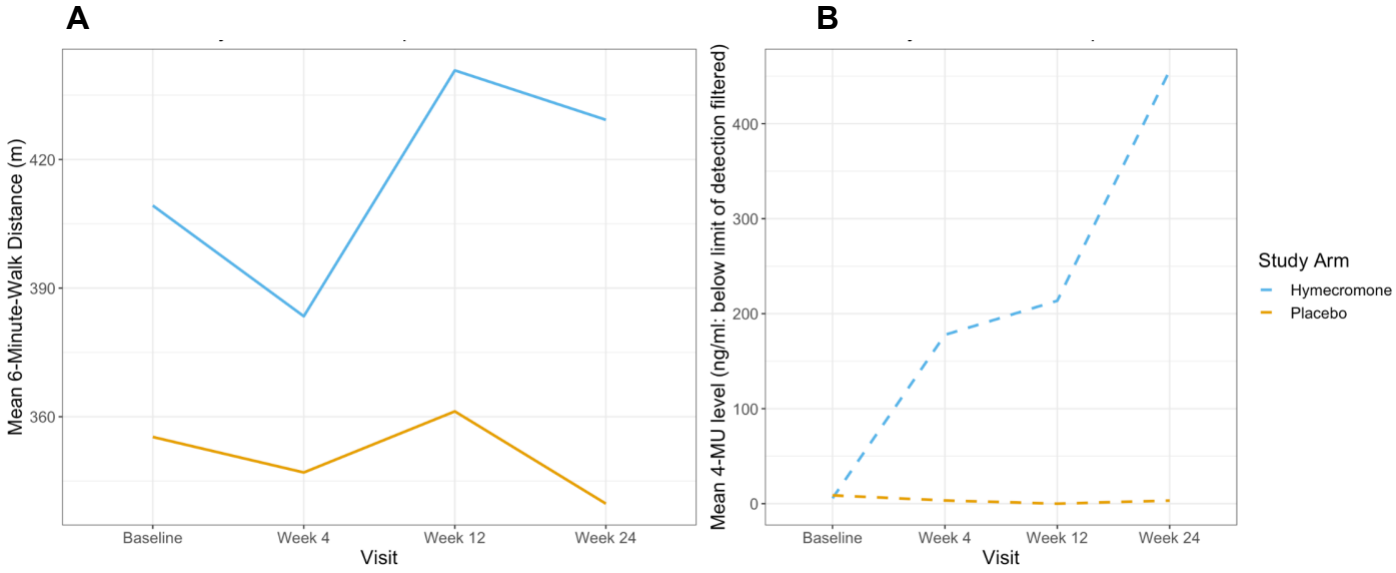
SUPPLEMENTAL

Supplemental Table 7. Summary of Pulmonary Function Test results (Group 3 PH-ILD), reported as mean (SD).

	Hymecromone (N=5)		Placebo (N=3)	
Timepoint	**Baseline**	**Week 24**	**Baseline**	**Week 24**
% Predicted FEV_1	78.8 (12.1)	80.6 (13.7)	69.7 (11.0)	74.0 (17.0)
% Predicted FVC	77.2 (13.4)	81.6 (13.7)	78.3 (14.8)	76.5 (17.7)
% Predicted TLC	89.0 (5.35)	87.8 (7.76)	94.0 (2.83)	NA
% Predicted DLCO, mL/min/mmHg	53.8 (8.41)	51.0 (2.74)	28.3 (8.08)	NA

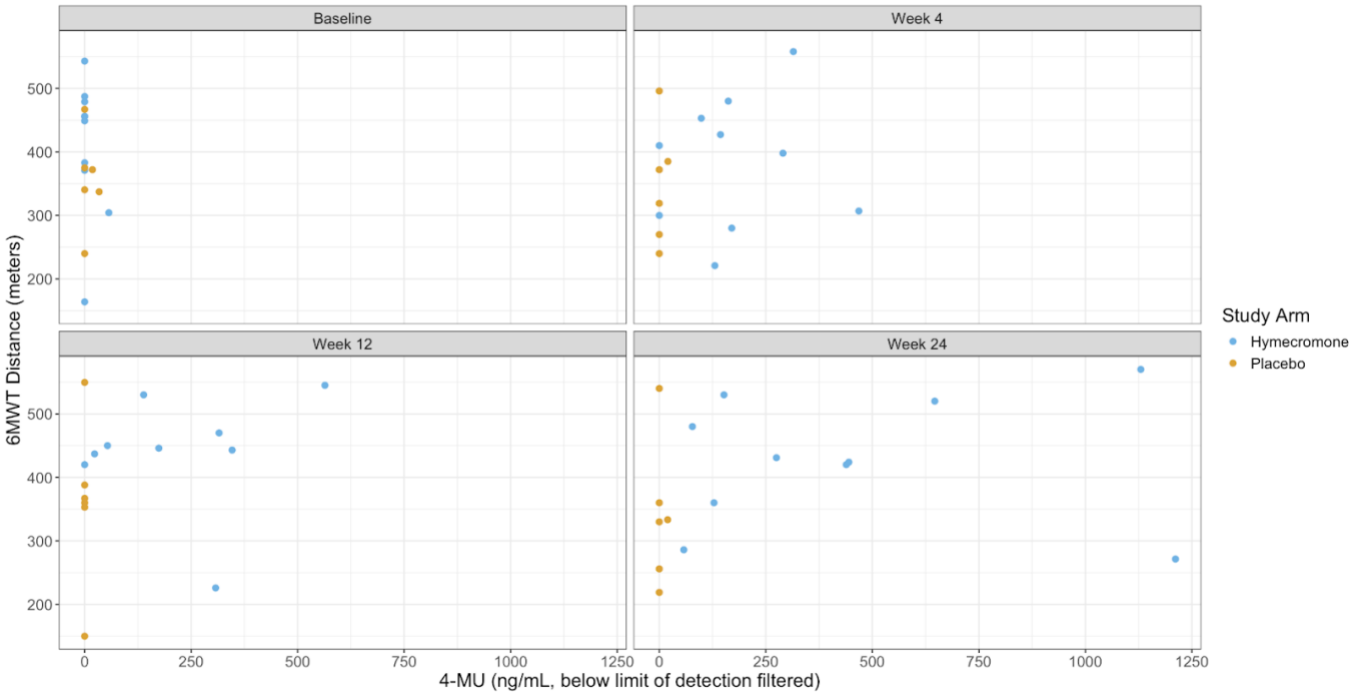
FEV_1 = forced expiratory volume in one second, FVC = forced vital capacity, TLC = total lung capacity, DLCO = diffusing capacity of the lung for carbon monoxide, NA = not applicable (due to 66.7% missingness).

SUPPLEMENTAL



Supplemental Figure 3. (A) Change in mean 6MWD by treatment group. Line plot showing change in mean 6-minute walk distance (m) from baseline to 24 weeks. In the hymecromone group (N=10), the mean 6-minute walk distance increased from 409 to 429 m. In the placebo group (N=6), the mean 6-minute walk distance decreased from 355 to 340 m. 6MWD = 6-minute walk distance test. **(B) Change in mean 4-MU concentration by treatment group.** Line plot showing change in 4-MU concentration (ng/mL) from baseline to 24 weeks amongst participants assigned to take hymecromone versus placebo. Blue dashed line indicates hymecromone and yellow dashed line indicates placebo. As expected, 4-MU concentration increases over time in the hymecromone arm. 4-MU = 4-methylumbelliferone. 4-MU is the chemical compound name for hymecromone.

SUPPLEMENTAL



Supplemental Figure 4. 6MWT distance versus 4-MU concentration by visit. Scatter plot showing individual 6-minute walk distance (m) versus 4-MU concentration (ng/mL) by discrete visit from baseline to 24 weeks. 6MWD = 6-minute walk distance test, 4-MU = 4-methylumbelliferone. 4-MU is the chemical compound name for hymecromone.

SUPPLEMENTAL

Supplemental Table 8. Summary of Drug Levels (combined Group 1 and Group 3)

	Hymecromone (N=10)		Placebo (N=6)	
Drug levels (ng/ml)	**Baseline**	**Week 24**	**Baseline**	**Week 24**
4-MU, mean (SD)	5.68 (18.0)	456 (421.0)	8.74 (14.4)	3.26 (8.0)
4-MUG, mean (SD)	0 (0.0)	3960 (2680.0)	0 (0.0)	0 (0.0)

4-MU = 4-methylumbelliferone. 4-MUG = 4-Methylumbelliferyl-β-D-glucuronide hydrate.
4-MU is the chemical compound name for hymecromone.

SUPPLEMENTAL

Supplemental Table 9. Patient demographics and baseline characteristics by PH group, N (%). Age is reported as sample median [minimum, maximum] due to skewed distributions.

	Group 1 PAH (N=8)	Group 3 PH-ILD (N=8)
Biological sex		
Female	7 (87.5)	5 (62.5)
Age, year	62.0 [51.5, 62.8]	63.5 [58.8, 66.5]
Race		
White	5 (62.5)	3 (37.5)
Black	0 (0)	1 (12.5)
Asian	3 (37.5)	3 (37.5)
Other	0 (0)	1 (12.5)
WHO PH Group		
Group 1 PAH	8 (100)	0 (0)
Group 3 PH-ILD	0 (0)	8 (100)
Background treatment for PH		
Monotherapy	0 (0)	2 (25)
Dual therapy	4 (50)	6 (75)
Triple therapy	4 (50)	0 (0)

PH = pulmonary hypertension, WHO = World Health Organization.

SUPPLEMENTAL

Supplemental Table 10. Summary of 6MWD, SpO2 and Borg Scale measurements (combined Group 1 PAH and Group 3 PH-ILD), reported as mean (SD).

	Hymecromone (N=10)				Placebo (N=6)			
Timepoint	**Screening**	**Week 4**	**Week 12**	**Week 24**	**Screening**	**Week 4**	**Week 12**	**Week 24**
6MWD, m	409 (110)	383 (104)	441 (91.1)	429 (101)	355 (73.5)	347 (92.1)	361 (127)	340 (112)
Number laps	13.2 (3.68)	12.5 (3.44)	14.2 (3.07)	13.8 (3.19)	11.5 (2.26)	11.2 (2.86)	11.7 (4.13)	12.0 (3.67)
Pre-6MWT SpO2	96.8 (2.35)	97.2 (1.75)	97.3 (1.58)	97.4 (2.22)	95.5 (3.67)	93.5 (3.89)	95.3 (4.37)	93.4 (3.29)
Peak-6MWT SpO2	93.4 (5.23)	92.4 (8.38)	91.9 (5.64)	89.5 (9.68)	88.7 (7.69)	85.3 (13.5)	87.0 (12.5)	86.8 (16.3)
Post-6MWT SpO2	96.0 (3.23)	96.0 (3.59)	96.8 (2.59)	96.6 (2.99)	93.8 (3.76)	93.7 (6.77)	93.8 (4.62)	95.2 (3.03)
Pre-6MWT Borg Scale	0.500 (0.972)	0.600 (0.843)	1.04 (1.98)	0.900 (1.29)	1.33 (1.97)	1.33 (1.51)	1.17 (0.983)	0.600 (0.894)
Peak-6MWT Borg Scale	3.50 (2.07)	3.00 (2.40)	4.28 (3.46)	4.25 (3.34)	4.33 (1.75)	5.17 (3.31)	3.83 (2.79)	3.60 (3.36)
Post-6MWT Borg Scale	0.600 (1.07)	1.00 (1.15)	1.33 (1.94)	0.850 (0.883)	1.83 (2.56)	2.67 (2.66)	1.83 (1.33)	0.800 (1.30)

6MWD = 6-minute walk distance, 6MWT = 6-minute walk test, SpO2 = peripheral oxygen saturation

SUPPLEMENTAL

Supplemental Table 11. Summary of 6MWD, SpO2 and Borg Scale measurements (Group 1 PAH), reported as mean (SD).

	Hymecromone (N=5)				Placebo (N=3)			
Timepoint	**Screening**	**Week 4**	**Week 12**	**Week 24**	**Screening**	**Week 4**	**Week 12**	**Week 24**
6MWD, m	449 (88.5)	440 (102)	419 (115)	423 (106)	392 (67.1)	418 (68.1)	435 (99.9)	376 (147)
Number laps	14.8 (2.95)	14.4 (3.36)	13.6 (3.85)	14.0 (3.54)	12.7 (2.08)	13.3 (2.31)	14.0 (3.46)	12.3 (5.13)
Pre-6MWT SpO2	95.8 (2.49)	96.4 (1.82)	97.6 (2.07)	97.4 (1.82)	93.3 (3.79)	93.3 (4.16)	93.7 (4.51)	92.3 (1.15)
Peak-6MWT SpO2	91.8 (5.36)	88.6 (10.2)	90.8 (7.43)	89.4 (8.35)	93.3 (3.06)	93.0 (3.61)	92.0 (2.00)	92.7 (0.577)
Post-6MWT SpO2	95.0 (2.74)	93.8 (3.70)	96.2 (3.42)	95.0 (3.08)	92.3 (4.73)	94.3 (4.51)	94.3 (6.03)	95.3 (3.06)
Pre-6MWT Borg Scale	0.800 (1.30)	0.600 (0.894)	0.600 (0.894)	1.20 (1.30)	0.667 (1.15)	1.67 (1.53)	1.00 (1.00)	1.00 (1.00)
Peak-6MWT Borg Scale	3.20 (1.92)	3.40 (2.88)	4.10 (4.19)	4.40 (4.04)	4.67 (2.08)	6.33 (3.21)	5.00 (3.61)	5.00 (3.61)
Post-6MWT Borg Scale	0.800 (1.30)	1.00 (1.22)	0.600 (0.894)	1.20 (1.10)	1.67 (2.08)	3.00 (1.00)	1.67 (0.577)	1.33 (1.53)

6MWD = 6-minute walk distance, 6MWT = 6-minute walk test, SpO2 = peripheral oxygen saturation

SUPPLEMENTAL

Supplemental Table 12. Summary of 6MWD, SpO2 and Borg Scale measurements (Group 3 PH-ILD), reported as mean (SD).

	Hymecromone (N=5)				Placebo (N=3)			
Timepoint	**Screening**	**Week 4**	**Week 12**	**Week 24**	**Screening**	**Week 4**	**Week 12**	**Week 24**
6MWD, m	369 (123)	327 (77.9)	468 (51.6)	435 (107)	318 (70.1)	276 (39.9)	288 (119)	303 (74.3)
Number laps	11.6 (3.91)	10.6 (2.51)	15.0 (2.00)	13.6 (3.21)	10.3 (2.08)	9.00 (1.00)	9.33 (3.79)	11.5 (0.707)
Pre-6MWT SpO2	97.8 (1.92)	98.0 (1.41)	97.0 (0.816)	97.4 (2.79)	97.7 (2.31)	93.7 (4.51)	97.0 (4.36)	95.0 (5.66)
Peak-6MWT SpO2	95.0 (5.15)	96.2 (4.32)	93.3 (2.63)	89.6 (11.9)	84.0 (8.54)	77.7 (16.3)	82.0 (17.6)	78.0 (28.3)
Post-6MWT SpO2	97.0 (3.67)	98.2 (1.79)	97.5 (1.00)	98.2 (2.05)	95.3 (2.52)	93.0 (9.64)	93.3 (4.04)	95.0 (4.24)
Pre-6MWT Borg Scale	0.200 (0.447)	0.600 (0.894)	1.60 (2.94)	0.600 (1.34)	2.00 (2.65)	1.00 (1.73)	1.33 (1.15)	0 (0)
Peak-6MWT Borg Scale	3.80 (2.39)	2.60 (2.07)	4.50 (2.89)	4.10 (2.97)	4.00 (1.73)	4.00 (3.61)	2.67 (1.53)	1.50 (2.12)
Post-6MWT Borg Scale	0.400 (0.894)	1.00 (1.22)	2.25 (2.63)	0.500 (0.500)	2.00 (3.46)	2.33 (4.04)	2.00 (2.00)	0 (0)

6MWD = 6-minute walk distance, 6MWT = 6-minute walk test, SpO2 = peripheral oxygen saturation

SUPPLEMENTAL

Supplemental Table 13. Metabolic Assessments, N (%) or mean (SD).

	Hymecromone (N=9 [A])		Placebo (N=6)	
Timepoint	**Baseline**	**Week 24**	**Baseline**	**Week 24**
Change in fasting glucose	88.1 (8.1)	87.7 (7.1)	99.0 (13.3)	97.2 (12.0)
Group 1	92.0 (8.4)	87.8 (6.8)	101 (18.3)	99.7 (4.0)
Group 3	85.0 (7.0)	87.6 (8.2)	97.0 (9.6)	94.7 (18.0)

[A] Excludes patient 6 in Group 1 PAH, as patient was non-fasting at time of baseline glucose measurement.

SUPPLEMENTAL

Supplemental Table 14. Summary of Exploratory Endpoints (combined and stratified by Group 1 and Group 3), N (%) or mean (SD).

	Hymecromone (N=10)		Placebo (N=6)	
Timepoint	**Baseline**	**Week 24**	**Baseline**	**Week 24**
Exploratory endpoint				
Change in ESR	20.8 (13.6)	23.3 (20.8)	10.0 (6.3)	13.2 (10.6)
Group 1	13.0 (9.0)	11.4 (6.7)	4.7 (4.6)	13.0 (15.1)
Group 3	28.6 (13.4)	35.2 (24.0)	15.3 (1.2)	13.3 (7.4)
Change in CRP	2.9 (2.6)	2.6 (3.3)	14.7 (29.6)	4.3 (3.0)
Group 1	2.1 (2.1)	2.0 (1.8)	1.7 (2.2)	4.3 (4.4)
Group 3	3.8 (3.0)	3.3 (4.5)	27.6 (4.5)	4.3 (1.9)

ESR = erythrocyte sedimentation rate, CRP = C-reactive protein