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Original research

Randomised, placebo-controlled trial of oral hymecromone in adults with pulmonary hypertension

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ABSTRACT

Background Pulmonary hypertension (PH) is a progressive cardiopulmonary condition associated with increased morbidity and mortality. The extracellular matrix component hyaluronan (HA) is linked to vascular remodelling and interstitial fibrosis in PH. We hypothesised that inhibition of HA synthesis with hymecromone could serve as a reverse-remodelling therapy in PH.

Methods We performed a proof-of-concept phase IIa randomised, double-blind, placebo-controlled study in adults with pulmonary arterial hypertension and PH associated with interstitial lung disease (PH-ILD). Patients were randomised to a 5:3 ratio and stratified by PH group to receive oral hymecromone or placebo two times per day over 24 weeks. The primary endpoint was change in pulmonary vascular resistance (PVR).

Results We enrolled 16 patients with PH with a median age of 62.0 years. There were no treatment-related adverse events leading to hymecromone discontinuation. No statistically significant difference in PVR was observed at 24 weeks for the experimental group compared with the placebo group (mean difference 0.61 Wood unit, 95% CI −1.5 to 2.7). Five patients with PH-ILD treated with hymecromone demonstrated an unadjusted absolute mean increase in 6 min walk distance of 66 m (SD 69.6) from baseline to 24 weeks and improvements in quality-of-life measures.

Conclusion Our exploratory analyses suggest that treatment with hymecromone could lead to improvements in clinically meaningful functional parameters in patients with PH-ILD. Further investigations in larger patient cohorts are warranted.

Trial registration number ClinicalTrials.gov: NCT05128929.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Hymecromone inhibits hyaluronan synthesis and is beneficial in multiple preclinical mouse models of pulmonary hypertension (PH) with measurable attenuation of vascular remodelling. Oral hymecromone (up to 3600 mg per day) is well tolerated in healthy participants. This pilot study was necessary to explore and characterise the effects of hymecromone in a small subset of patients with PH.

WHAT THIS STUDY ADDS

⇒ Hymecromone is safe and well tolerated in adults with pulmonary arterial hypertension and PH associated with interstitial lung disease (PH-ILD).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Novel hymecromone therapy could lead to improvements in 6 min walk distance and quality-of-life metrics in patients with PH-ILD, though further investigations in larger patient cohorts are needed.

INTRODUCTION

Pulmonary hypertension (PH) is a cardiopulmonary condition associated with increased pulmonary arterial pressures that often leads to progressive right ventricular hypertrophy, right-sided heart failure, decreased quality of life and death.[1 2] According to the WHO classification system, there are five groups of PH.[3] These include group 1 patients, who have pulmonary arterial hypertension (PAH), and group 3 patients, who have underlying lung diseases and hypoxia.[4] Both groups are associated with severe exercise intolerance and increased mortality.[5 6]

Current treatment options for PH are limited in terms of number of approved treatments and duration of effects of those treatments. Hyaluronan (HA), a glycosaminoglycan of the extracellular matrix, is essential for maintaining healthy pulmonary tissue structure and function. Under states of inflammation, a fragmented form of HA predominates and is implicated in the development of PH.[7] In preclinical models of PH, HA is associated with proliferation of pulmonary artery smooth muscle cells and aberrant vascular remodelling.[8] In humans, elevated HA levels have been reported in several chronic lung diseases including PAH.[9–11] In addition, increased HA levels have also been reported in autoimmune diseases such as systemic sclerosis linked to connective tissue disease, in interstitial lung disease (ILD)[12] and in PH-related lung diseases including obstructive pulmonary disease and pulmonary fibrosis.[9–11 13]

Collectively, these studies point to a possible pathogenic role for HA in PH and suggest that therapies targeting HA may serve as novel reverse-remodelling treatments in the disease.

Linked

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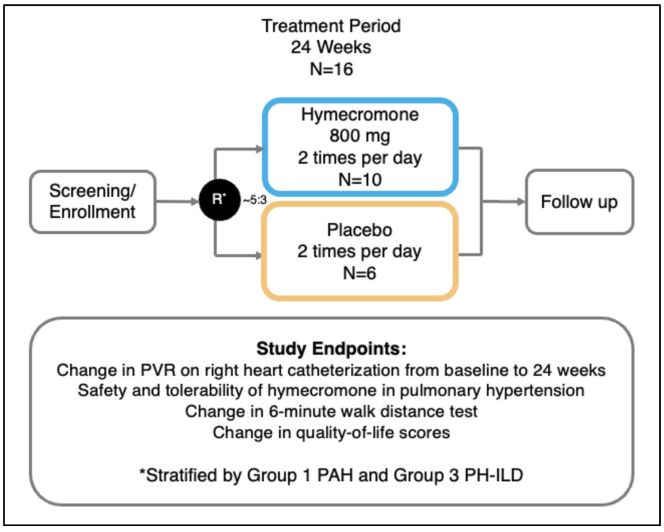


Figure 1 Overview of study design. 16 eligible participants were randomised to hymecromone or placebo in a 5:3 ratio using a computer-generated randomisation scheme, stratified by PH group. Once assigned to the treatment arm, participants were on treatment continuously for 24 weeks. PAH, pulmonary arterial hypertension; PH-ILD, pulmonary hypertension associated with interstitial lung disease; PVR, pulmonary vascular resistance.

Oral hymecromone, also known as 4-methylumbelliferone (4-MU), is a small molecule inhibitor of HA synthesis.[14 15] 4-MU mainly functions as a competitive substrate for uridine 5'-diphospho-glucuronosyltransferase, an enzyme involved in HA synthesis. 4-MU is of benefit in multiple models of lung disease,[16–18] including in multiple preclinical mouse models of PH with measurable attenuation of vascular remodelling.[8 19] In addition, 4-MU has demonstrated antifibrotic activity in other settings of inflammation and fibrosis.[20–22] The oral drug, hymecromone, is approved (European Union reference date 27 July 1965) for treatment of biliary dyskinesia. Our group has previously demonstrated that oral hymecromone (up to 3600 mg per day) was well tolerated in healthy participants and was effective at decreasing levels of HA in the sputum.[23] It has not previously been shown if hymecromone is safe and effective in patients with PH and underlying lung fibrosis.

We hypothesised that oral hymecromone, a safe, well-tolerated drug in other patient populations, will be safe in PH and serve as an effective antifibrotic and reverse-remodelling agent in group 1 PAH and group 3 PH associated with ILD (PH-ILD) individuals. To better evaluate the safety and tolerability of hymecromone over 24 weeks in adults with PH, we performed a phase IIa proof-of-concept trial. We designed this study as a prospective, randomised, double-blind, placebo-controlled trial to investigate the effect of oral hymecromone at doses of 1600 mg daily on pulmonary haemodynamics and other functional measures of PH disease progression.

METHODS

Study design

In this prospective, randomised, double-blind, placebo-controlled, phase IIa study, adult patients with PH were randomised to receive two times per day dosing of oral hymecromone or dose-matched placebo (ClinicalTrials.gov number NCT: 05128929) over a treatment period of 24 weeks. We conducted this study at a single, tertiary care centre in the USA between 1 April 2022 (first patient assessment) and 26 September 2023 (last patient follow-up assessment). We concluded the study on completion of the final patient assessment. We randomised 16 patients in a 5:3 ratio to hymecromone (n=10) and placebo (n=6) (figure 1), stratified by PH group.

Table 1 Patient demographics and baseline characteristics for all randomised patients, n (%) or mean (SD)

	Hymecromone (n=10)	Placebo (n=6)
Biological sex		
Female	8 (80.0)	4 (66.7)
Age, year	57.0 (24.0, 66.0)	66.5 (62.0, 71.0)
Race		
White	3 (30.0)	5 (83.3)
Black	0 (0.0)	1 (16.7)
Asian	6 (60.0)	0 (0.0)
Other	1 (10.0)	0 (0.0)
BMI, kg/m^2	25.9 (5.5)	28.0 (8.9)
WHO PH group		
Group 1 PAH	5 (50.0)	3 (50.0)
Group 3 PH-ILD	5 (50.0)	3 (50.0)
WHO functional class*		
I	0 (0.0)	0 (0.0)
II	7 (70.0)	4 (66.7)
III	3 (30.0)	2 (33.3)
IV	0 (0.0)	0 (0.0)
Cause of lung disease		
Interstitial lung disease	5 (50.0)	3 (50)
Connective tissue disease	3 (30.0)	0 (0)
Drug associated	1 (10.0)	1 (16.7)
Idiopathic	1 (10.0)	2 (33.3)
Background treatment for PH		
Monotherapy	0 (0)	2 (33.3)
Dual therapy	8 (80)	2 (33.3)
Triple therapy	2 (20)	2 (33.3)
Haemodynamic variables		
mPAP, mm Hg	43.6 (10.0)	47.5 (9.40)
PCWP, mm Hg	11.6 (3.17)	12.5 (2.88)
Cardiac output, L/min	4.30 (1.00)	4.81 (1.16)
PVR,† Wood units	7.97 (2.65)	8.23 (4.16)
Pulmonary function test		
% predicted FEV_1	86.2 (15.7)	72.2 (8.84)
% predicted FVC	85.8 (16.9)	77.3 (12.1)
% predicted TLC	91.9 (13.5)	91.6 (4.72)
% predicted DLCO, mL/min/mm Hg	62.8 (28.7)	57.7 (32.6)
6MWD		
Baseline distance, m	409 (110)	355 (73.5)

Age is reported as sample median (minimum, maximum) due to skewed distributions.
*The WHO functional class ranges from I to IV with higher numbers indicating greater functional limitations. Only patients in WHO functional class II and III were included in this study.
†The mean PVR is calculated using the thermodilution method.
BMI, body mass index; DLCO, diffusing capacity of the lung for carbon monoxide; FEV_1, forced expiratory volume in one second,; FVC, forced vital capacity; mPAP, mean pulmonary artery pressure; 6MWD, 6 min walk distance; PAH, pulmonary arterial hypertension; PCWP, pulmonary capillary wedge pressure; PH, pulmonary hypertension; PH-ILD, pulmonary hypertension associated with interstitial lung disease; PVR, pulmonary vascular resistance; TLC, total lung capacity.

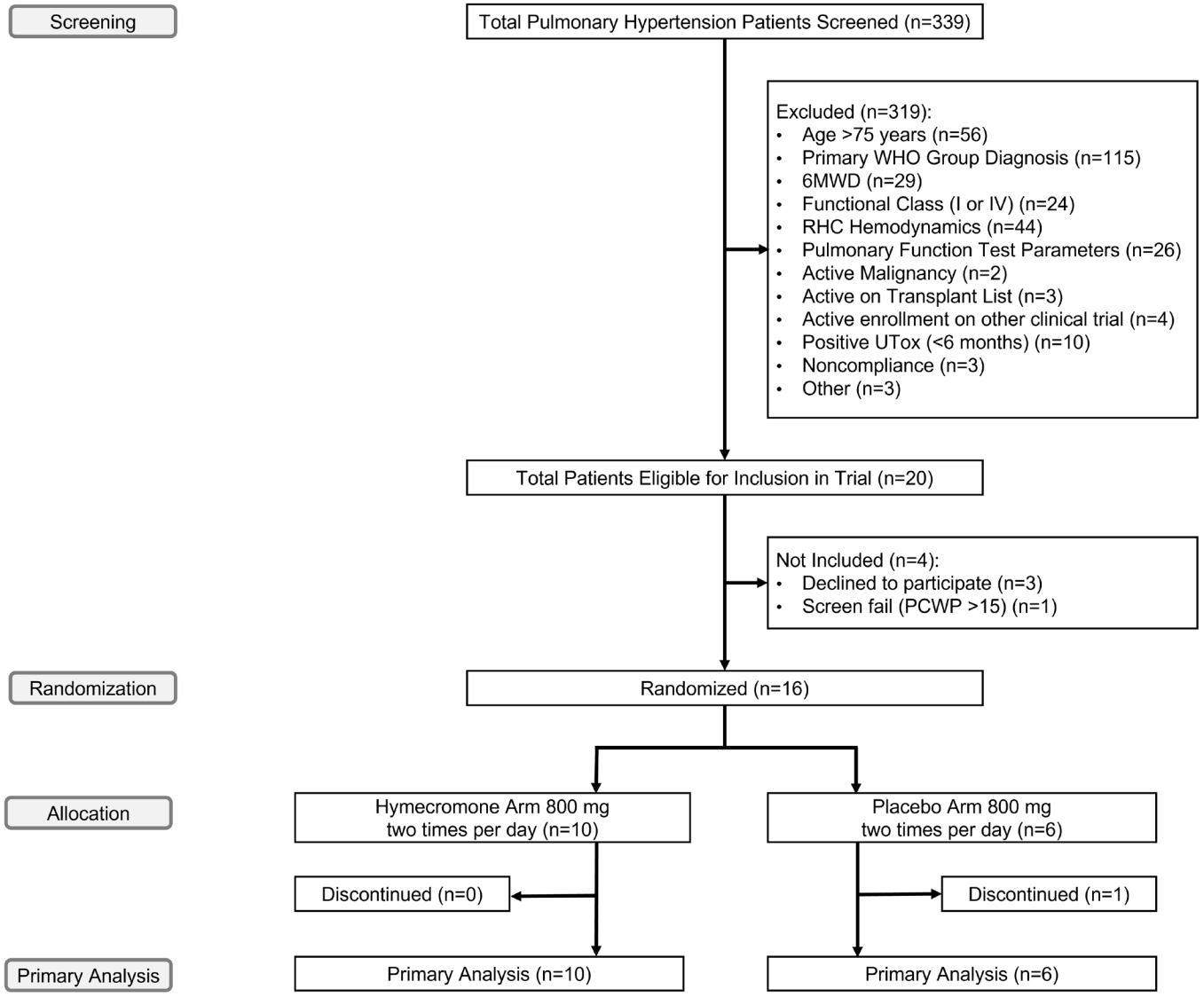


Figure 2 Consolidated Standards of Reporting Trials diagram. Flow of participants through screening, randomisation, allocation and primary analysis. A total of 20 out of 339 patients with pulmonary hypertension were eligible for inclusion in the trial. 16 patients were randomised to hymecromone (n=10) and placebo (n=6). All 16 patients were included in the primary analysis. 6MWD, 6 min walk distance; PCWP, pulmonary capillary wedge pressure; RHC, right heart catheterisation; UTox, urine toxicology screen.

Enrolment

This trial enrolled male and female patients with PAH and PH-ILD based on the hypothesised utility of hymecromone in addressing PH-related vascular remodelling and fibrosis.[1] Enrolled patients underwent a right heart catheterisation (RHC) at randomisation and were eligible for inclusion if they had a mean pulmonary arterial pressure (mPAP) of ≥25 mm Hg, pulmonary vascular resistance (PVR) ≥400 dynes·s/cm^5 and pulmonary capillary wedge pressure (PCWP) ≤20 mm Hg. Online supplemental figure 1 shows the distribution of PCWPs for all participants at time of screening. To avoid the 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. Eligible patients were functional class II–IV and were stable on background therapy for PH for at least 90 days prior to enrolment. This trial was enriched for patients with autoimmune disease as summarised in online supplemental table 1. A full list of inclusion and exclusion criteria is available in online supplemental table 2.

Study approval

This single-site trial was conceived, designed, initiated and performed by the academic investigators with RTZ serving as sponsor. The trial was initiated following approval of an investigational new drug application by the US Food and Drug Administration. The trial was conducted in alignment with good clinical practice standards, applicable laws and regulations. An independent data and safety monitoring committee provided guidance on patient safety. The Stanford Quantitative Sciences Unit conducted all statistical analyses. Halo Biosciences obtained drug product/placebo and provided support for regulatory procedures. The authors confirm the accuracy and completeness of the data and analysis as well as the adherence of trial conduct to the protocol.

Procedures

Eligible patients were recruited from Stanford Hospital and Clinics in Stanford, California, USA. An equal number of

Table 2 Summary of primary and secondary endpoints (combined group 1 PAH and group 3 PH-ILD), reported as mean (SD) or difference in means (95% CI)

	Hymecromone (n=10)		Placebo (n=6)		
Timepoint	Baseline	Week 24	Baseline	Week 24	Treatment effect*
Primary endpoint					
PVR, Wood units	7.97 (2.65)	7.27 (2.83)	8.23 (4.16)	7.11 (2.69)	0.61 (–1.5 to 2.7)
Secondary endpoints					
mPAP	43.6 (10.0)	40.2 (10.9)	47.5 (9.4)	42.4 (9.34)	1.47 (–4.3 to 7.3)
6MWD, m	409 (110)	429 (101)	355 (73.5)	340 (112)	47.26 (–21.4 to 116.0)
emPHasis-10	20.6 (9.9)	15.0 (7.6)	19.3 (12.2)	14.3 (10.5)	–0.12 (–8.2 to 8.0)
SGRQ	32.9 (13.3)	27.8 (9.9)	36.0 (17.4)	39.1 (27.1)	–9.00 (–19.4 to 1.4)
Serum HA	178 (68.0)	220 (195)	182 (31.7)	143 (38.6)	73.92 (–45.6 to 193.5)
NT-proBNP, pg/mL	677 (976)	618 (514)	531 (329)	336 (280)	231.47 (–377.3 to 840.2)

*Treatment effect estimate is adjusted for pulmonary hypertension group.
emPHasis-10, Evaluating Measurement of Pulmonary Hypertension Symptoms-10 Questionnaire; HA, hyaluronan; mPAP, mean pulmonary arterial pressur; 6MWD, 6 min walk distance; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAH, pulmonary arterial hypertension; PH-ILD, pulmonary hypertension associated with interstitial lung disease; PVR, pulmonary vascular resistance; SGRQ, St. George Respiratory Questionnaire.

patients had confirmed group 1 PAH and group 3 PH-ILD per WHO definitions.[1 4] Each arm was randomised in a 5:3 ratio (treatment: placebo) with treated patients receiving 800 mg oral hymecromone two times per day and placebo patients receiving two placebo tablets two times per day. Randomisation was conducted via a computer-generated scheme, stratified by PH type and managed by an unblinded biostatistician at the Stanford Quantitative Sciences Unit. A 4-week screening period was followed by 24 weeks of treatment and a 4-week follow-up period. The treatment period included clinical assessments at baseline, week 4, week 12 and week 24. Telephone or email assessments were performed at weeks 2, 8, 16 and 20. All assessments were screened for efficacy and safety. Adverse events (AEs) were documented in case report forms.

Clinical data capture and analysis

Case report forms and data entry were managed by the study team at the Vera Moulton Wall Center for Pulmonary Vascular Disease. Clinical laboratory tests were processed by the Stanford Anatomic Pathology and Clinical Laboratories. HA, 4-MU and 4-methylumbelliferyl glucuronide (4-MUG) levels were assessed by the Stanford Bollyky lab following protocols previously described.[23] Data acquisition and analysis were performed using Analyst, V.1.6.1, software (AB SCIEX).

Trial endpoints

The primary endpoint was the change from baseline to week 24 in PVR measured through RHC. Key secondary endpoints included (1) the safety and tolerability of hymecromone according to NIH Common Terminology Criteria for Adverse Events, (2) change in mPAP by RHC from baseline to week 24, (3) change in baseline to week 24 in 6 min walk distance (6MWD) and (4) change in quality of life. Quality of life was assessed via the St. George's Respiratory Questionnaire (SGRQ) and the Evaluating Measurement of Pulmonary Hypertension Symptoms-10 Questionnaire (emPHasis-10) health-related quality-of-life questionnaire. Exploratory endpoints of note included PH and inflammatory pharmacodynamic markers, pulmonary function tests and serum concentrations of 4-MU and 4-MUG. A complete list of endpoints is included in online supplemental table 3. The 6 min walk test (6MWT) protocol employed in this study is included in online supplemental table 4. All 6MWTs were performed in a standardised manner per the 6MWT protocol and ATS guidelines.[24]

Statistics

All analyses were conducted following the intention-to-treat (ITT) principle and included all randomised participants. Descriptive statistics, including proportions for categorical variables, means, medians, SD and IQR for continuous variables, were reported for baseline demographics and other patient characteristics. The prespecified analysis of the primary endpoint and secondary continuous endpoints used a constrained longitudinal data analysis model with random effects to account for intraparticipant correlation and covariate adjustment for PH group. The 95% CIs were calculated using the Satterthwaite approximation of the model df. In post hoc analyses, we estimated the Glass rank biserial correlation coefficient to present an effect size and p value for the primary endpoint using non-parametric methods in response to a reviewer. Additional details can be found in the statistical analysis plan provided in the online supplemental materials.

We conducted additional exploratory analyses using the same model described above to investigate the effect across subgroups, but given the small sample size, no analyses were powered to detect an effect. Participants were followed for AEs from the time of the first dose to either the date of the last dose or the RHC performed at 24 weeks, whichever occurred later. AEs were characterised by AE type and treatment arm.

Data availability

Statistical analyses were conducted using R V.4.0.2 (R Core Team (2022)) with the readr, table 1, kableExtra, dplyr, lme4, lmerTest and ggplot2 packages. Values for all data points in graphs are reported in the supporting data values file.

RESULTS

Study population

Following screening and informed consent, 16 patients were randomly assigned to receive hymecromone (n=10) or placebo (n=6) in addition to stable background therapy in this prospective, double-blind proof-of-concept study. Reasons for screening failure for the 319 excluded patients are detailed in figure 2. The median age for all participants was 62 years (age range 24–71). Most participants (87.5%) were on dual or triple therapy for PH with a prostacyclin class therapy and a nitric oxide pathway phosphodiesterase type 5 inhibitor. In accordance with prior specifications, we stratified treatment arms according to the WHO PH classification system. Table 1 summarises the demographic, clinical and haemodynamic characteristics of patients at baseline.

Clinical endpoints

The primary endpoint of this study was the change in PVR on RHC from baseline to week 24. We analysed a total of sixteen patients in the ITT population for the primary outcome. All but one patient underwent postintervention RHC assessment. The RHC was deemed clinically unsafe for one participant whose 24-week RHC data were not collected; however, their baseline data were included in the analysis. Another participant had their postintervention RHC performed early at 16 weeks because they were withdrawn from the study (due to a scheduled transplantation). No statistically significant difference in PVR was observed at 24 weeks for the experimental group compared with the placebo group (mean difference 0.61 WU, 95% CI −1.5 to 2.7) (table 2 and online supplemental table 5).

The secondary endpoints of this study included change in mPAP, 6MWD test and quality-of-life measures from baseline to week 24. Our analysis of secondary endpoints did not reveal any meaningful differences between the experimental and placebo arms (table 2).

We conducted exploratory subgroup analyses for PAH and PH-ILD participants for both our primary and secondary endpoints, as specified a priori, to assess whether response to hymecromone intervention differed by PH grouping. Among patients with PH-ILD, we observed an increase from baseline in the 6MWD test and improvement in quality-of-life measures despite no significant change in PVR (online supplemental figure 2). From baseline to week 24, patients with PH-ILD on hymecromone (n=5) experienced a mean increase of 66 m (SD 69.6) while their emPHasis-10 and SGRQ scores decreased by 9.8 (SD 10.4) and 12.4 (SD 3.5), respectively (table 3). The placebo-corrected change for 6MWD for these five participants was 82 m (66 m increase in n=5 treated vs 16 m decrease in n=3 placebo). We also visualised change in 6MWD and emPHasis-10 scores in all participants using spaghetti plots (figure 3). We observed that four out of the five participants with PH-ILD on hymecromone demonstrated an increase in total distance

Table 3 Summary of primary and secondary endpoints among group 1 PAH participants (part A) and group 3 PH-ILD participants (part B), mean (SD)

	Hymecromone (n=5)			Placebo (n=3)		
Timepoint	**Baseline**	**Week 24**	**Delta**	**Baseline**	**Week 24**	**Delta**
A						
Primary endpoint						
PVR, Wood units	9.1 (3.5)	8.6 (3.2)	−0.5 (1.8)	5.9 (0.8)	5.2 (0.7)	−0.4 (1.4)
Secondary endpoints						
mPAP	50.4 (7.9)	48.0 (8.2)	−2.4 (4.0)	45.3 (6.1)	46.0 (8.5)	0 (0)
6MWD, m	449 (88.5)	423 (106)	−26 (31.3)	392 (67.1)	376 (147)	−16 (95.0)
emPHasis-10	16.2 (9.0)	14.8 (10.0)	−1.4 (6.8)	17.3 (14.2)	14.0 (10.0)	−3.3 (6.5)
SGRQ	22.0 (6.3)	24.0 (11.7)	2.0 (9.3)	32.6 (14.8)	31.7 (18.3)	−0.9 (5.0)
Serum HA	172 (95.6)	160 (75.1)	−14.5 (38.9)	189 (34.4)	169 (14.0)	−21.8 (50.5)
NT-proBNP, pg/mL	284 (223)	389 (455)	105 (347)	396 (368)	305 (243)	−91.3 (134)
B						
Primary endpoint						
PVR, Wood units	6.8 (1.0)	6.1 (1.8)	−0.8 (1.1)	10.4 (5.2)	8.4 (2.8)	−2.0 (3.2)
Secondary endpoints						
mPAP	36.8 (6.9)	32.4 (7.0)	−4.4 (0.5)	49.7 (13.0)	40.0 (10.8)	−9.7 (9.1)
6MWD, m	369 (123)	435 (107)	66.0 (69.6)	318 (70.1)	303 (74.3)	−16 (5.3)
emPHasis-10	25.0 (9.7)	15.2 (5.6)	−9.8 (10.4)	21.3 (12.1)	14.7 (13.2)	−6.7 (7.0)
SGRQ	43.9 (7.6)	31.5 (7.0)	−12.4 (3.5)	39.3 (22.5)	46.5 (36.6)	7.2 (14.1)
Serum HA	185 (33.9)	281 (266)	95.8 (243)	175 (34.3)	117 (38.8)	−51.3 (29.2)
NT-proBNP, pg/mL	1070 (1310)	846 (506)	−225 (1150)	666 (283)	367 (366)	−299 (649)

emPHasis-10, Evaluating Measurement of Pulmonary Hypertension Symptoms-10 Questionnaire; HA, hyaluronan; mPAP, mean pulmonary arterial pressure; 6MWD, 6 min walk distance; NT-proBNP, N-terminal prohormone of brain natriuretic peptide; PAH, pulmonary arterial hypertension; PH-ILD, pulmonary hypertension associated with interstitial lung disease; PVR, pulmonary vascular resistance; SGRQ, St. George Respiratory Questionnaire.

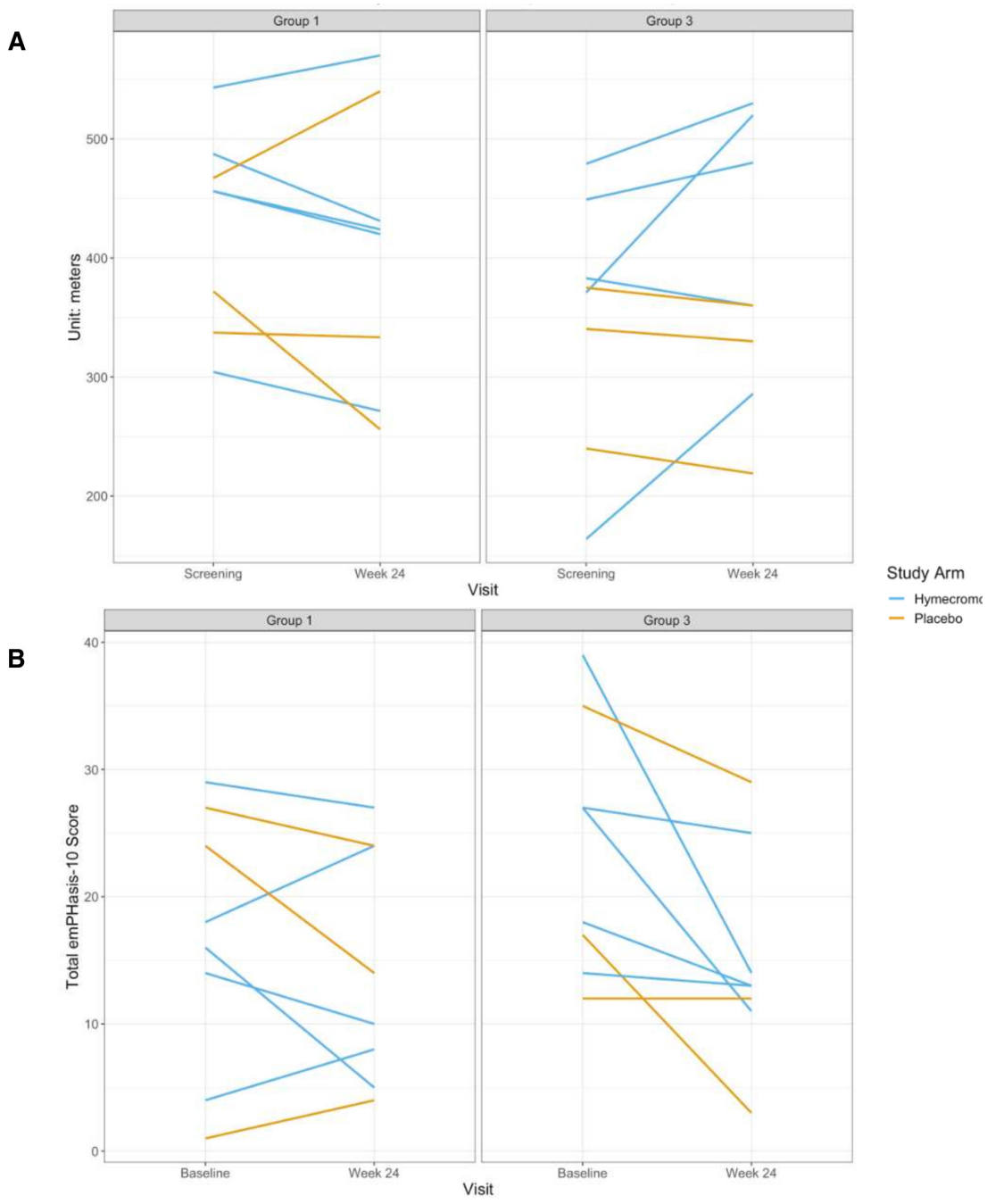


Figure 3 Change in 6MWD and total emPHasis-10 Score by treatment group and PH group. (A) Line plot showing change in 6 min walk distance (m) from screening to 24 weeks, stratified by group 1 pulmonary arterial hypertension (PAH) and group 3 pulmonary hypertension associated with interstitial lung disease (PH-ILD) participants. (B) Line plot showing change in total emPHasis-10 Score from baseline to 24 weeks, stratified by group 1 PAH and group 3 PH-ILD participants. 6MWD, 6 min walk distance; emPHasis-10, Evaluating Measurement of Pulmonary Hypertension Symptoms-10 Questionnaire; PH, pulmonary hypertension.

walked (figure 3A) and a decrease in total emPHasis-10 score over 24 weeks (figure 3B). The placebo-corrected change for total emPHasis-10 score for these five participants was −3.1 (9.8-point decrease in n=5 treated vs 6.7-point decrease in n=3 placebo). The placebo-corrected change for SGRQ Score for these five participants was −19.6 (12.4-point decrease in n=5 treated vs 7.2-point increase in n=3 placebo). These results suggest that hymecromone provided a symptomatic benefit in patients with PH-ILD, but this was not associated with improvements in PVR.

Safety and tolerability

Patients tolerated the hymecromone intervention well. All AEs are summarised in table 4. AEs occurred in 63.5% (10/16) of patients overall. The type and frequency of AEs were similar across experimental and control arms. There were two treatment-related AEs in this trial: fatigue (n=1) and diarrhoea (n=1). Both events were deemed to be 'possibly related' to the treatment by the investigators and affected the same subject enrolled in the placebo arm only. Only one serious AE (SAE) was reported in the experimental arm. The data safety and

Table 4 Quantitative summary of AEs

	Hymecromone (n=10)	Placebo (n=6)
Total number of AEs, n	5	7
Average number of AE per participant, n	0.5	1.2
AE treatment relatedness per participant, n	0	1*
Total number of SAEs, n	1	3
Average number of SAE per participant, n	0.1	0.5
Discontinuations due to SAE	0	1
Deaths due to SAE	0	0
AE, n		
Hypoxia	1	1
Lung infection	2	0
Pneumonitis	1	1
Diarrhoea	0	2†
Chest pain (cardiac)	1	0
Creatinine increased	0	1
Thromboembolic event	0	1
Fatigue	0	1†

*Deemed by investigators to be 'possibly related' to treatment assignment and occurred in the same individual participant.
†Reported symptom deemed 'possibly related' to the assigned treatment.
AE, adverse event; SAE, serious adverse event.

monitoring board determined that this SAE was not related to the hymecromone treatment, and the SAE resolved without sequelae. Hymecromone treatment was neither interrupted nor discontinued. Three patients assigned to placebo reported SAEs. One patient in the placebo arm terminated from the trial prematurely due to clinical worsening of hypoxia. These safety results indicate that hymecromone was generally safe and well tolerated in this population.

DISCUSSION

Our study shows that oral hymecromone is safe and well tolerated in patients with PAH and PH-ILD. There were no hymecromone treatment-related AEs in this study and no treatment-related discontinuations. Hymecromone (1600 mg/day) use over 24 weeks had no statistically significant effect on PVR measured by RHC when compared with placebo.

Table 5 Summary of pulmonary function test results (combined group 1 PAH and group 3 PH-ILD), reported as mean (SD)

	Hymecromone (n=10)		Placebo (n=6)	
Timepoint	Baseline	Week 24	Baseline	Week 24
% predicted FEV_1	86.2 (15.7)	87.5 (14.6)	72.2 (8.8)	73.4 (10.6)
% predicted FVC	85.8 (16.9)	88.9 (15.1)	77.3 (12.1)	76.4 (13.0)
% predicted TLC	91.9 (13.5)	92.9 (11.6)	91.6 (4.7)	91.0 (5.7)
% predicted DLCO, mL/min/mm Hg	62.8 (28.7)	61.1 (31.6)	57.7 (32.6)	73.3 (28.3)

PFT results separated by PH group are included in the online supplemental materials.
DLCO, diffusing capacity of the lung for carbon monoxide; FEV_1, forced expiratory volume in one second; FVC, forced vital capacity; PAH, pulmonary arterial hypertension; PH-ILD, pulmonary hypertension associated with interstitial lung disease; TLC, total lung capacity.

Our exploratory analyses suggest that response to hymecromone intervention may differ by PH grouping. We were intrigued that patients with PH-ILD on hymecromone demonstrated an unadjusted absolute mean increase in 6MWD of 66 m (SD 69.6)—constituting a median increase of 97 m from baseline to 24 weeks. Given the small sample size (n=5 treated vs n=3 placebo), this comparison was not statistically powered. However, it is possible that hymecromone use had a beneficial effect on symptoms specifically in PH-ILD. We note that patients with PH-ILD also demonstrated improvements in quality-of-life measures: the emPHasis-10 and the SGRQ.

While a number of patients in this proof-of-concept trial are too small to draw statistically meaningful conclusions, our 6MWD results are promising. A recent meta-analysis demonstrated that the minimal clinically significant change in 6MWD is a difference of 33 m in adults with PAH.[25] Our trial therefore provides preliminary evidence that hymecromone may have a clinically beneficial effect in patients with PH-ILD. It is likely that the observed improvement in the 6MWT is specifically noticeable in the group 3 PH-ILD patients because, as a cohort, these patients had substantially lower 6MWT measures at baseline. Larger studies are needed to assess for a statistically significant improvement in the 6MWT and quality-of-life measures, as was observed in our treated PH-ILD subgroup in this study.

It is notable that any functional improvement which occurred in patients with PH-ILD on hymecromone in this study was not associated with statistically significant changes in PVR, mPAP or pulmonary function. We acknowledge that this finding conflicts with expected haemodynamic changes seen with other therapeutics used in PH-ILD.[26] PFT results for enrolled patients are included in table 5 and online supplemental tables 6 and 7. We have two possible hypotheses for this outcome, which warrant further exploration.

We speculate that hymecromone may have beneficial off-target effects on exercise capacity and insulin tolerance. Multiple preclinical studies have demonstrated metabolic benefits of 4-MU through preserved glycaemic control,[27] activation of brown adipose tissue[28] and mitochondrial respiration.[29] Our preliminary data suggest that there may be improvement in exercise tolerance and symptoms in this blinded and randomised trial. We hypothesise that through improved metabolic effects, there may be improved musculoskeletal function and that future studies should consider the assessment of muscle biopsy or validated functional measures to better understand the effect of hymecromone in patients with PH-ILD.

Additionally, we did not observe a decrease in serum HA in this study (table 2); this is consistent with what we observed in our recent phase I study in healthy controls, where sputum, but not serum HA, decreased on hymecromone.[23] Unfortunately, we were unable to collect sputum in this study due to safety concerns. It may be that serum HA levels reflect extracellular matrix turnover and do not correlate with changes in the lung per se. The therapeutic impact of hymecromone may have been limited by its relatively poor pharmacokinetics. While oral hymecromone is essentially 100% absorbed, it undergoes extensive first-pass metabolism in the liver, resulting in only ~3% of the drug being systemically available.[30] When hymecromone is dosed intravenously, which allows for the bypass of first-pass metabolism, systemic exposures observed are greater than 100-fold higher as compared with oral dosing.[31 32] This poor oral bioavailability presents a challenge in the development of hymecromone as a therapeutic agent, suggesting that there may be opportunities for reformulation to enhance its clinical profile.

A strength of this randomised clinical trial design is the use of enrichment strategies in building a targeted study population based on disease characteristics and the hypothesised mechanism of hymecromone.[33] Our inclusion and exclusion criteria were relatively selective to enrol individuals with PAH and PH-ILD and WHO functional classes II–IV. As previously stated, HA has been implicated in the pathogenesis of several autoimmune diseases, and so we also enriched for patients with concomitant autoimmunity (online supplemental table 1). Due to the rarity of PH and challenges with patient recruitment, early phase II trials often have wide inclusion and exclusion criteria, which can make interpretation of effect difficult given the heterogeneity of PH aetiology.[34]

A major limitation of this work is the study's small sample size, which limits our power to detect differences in efficacy between hymecromone and placebo and could result in a type 1 error. As such, the results of this study should be interpreted with caution. In any clinical trial investigating a rare disease entity with clinical heterogeneity such as PH, there is a risk that group mean data hide non-responders whose negative data are matched by super-responders.[34] A separate limitation is that, due to operational constraints, we were not able to conduct controlled pharmacokinetic analyses, making the interpretation of 4-MU and 4-MUG levels challenging (online supplemental figures 3 and 4 and online supplemental table 8). Related operational constraints are that we did not have dose-response data to inform optimal drug exposures, and we were only able to study a single dose in this trial.

The effect of hymecromone we observed on the 6MWD and quality-of-life measures in PH-ILD warrants further investigation in a larger patient cohort. Given the diversity of PH, this pilot study was necessary to explore and characterise the effects of hymecromone in a small subset of disease.[34] Patient demographics and baseline characteristics by PH group, a summary of 6MWD, SpO_2 and Borg scale measurements, and exploratory assessments of metabolic and inflammatory changes are summarised in online supplemental tables 9–14, respectively. Mirroring other trials in PH,[35] a future trial investigating oral hymecromone in PH-ILD should use the 6MWD as the primary end point.

In conclusion, our findings suggest that oral hymecromone was safe and well tolerated in patients with PH in this small proof-of-concept trial and support the pursuit of larger clinical trials investigating the symptomatic benefits of hymecromone in adult patients with PH-ILD.

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Contributors RTZ, VdJP, NN, AK and PLB designed the research study. TP, VdJP and RTZ conducted the clinical trial. KC, AK and PLB wrote the first draft of the manuscript. BG and HH acquired and analysed the data. KC, NN, AK, TP, ARF, HK-Q, BG, HH, GK, SDL, JIR, PLB, VdJP and RTZ provided data interpretation and manuscript revisions. KC, VdJP and RTZ wrote the final version of the manuscript. KC and NN share the first author position, which reflects their equivalent contributions to this work. RTZ is the guarantor.

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Competing interests NN, GK and PLB have filed intellectual property around 4-methylumbelliferone (4-MU). NN, AK and PLB are cofounders, SDL is a consultant, and ARF, HK-Q, GK, JIR, VdJP and RTZ are advisors to Halo Biosciences, a company that is developing 4-MU for various indications. Halo Biosciences is a spin-out from Stanford University focused on advancing the clinical development of 4-MU. At the time the SATURN study was conceived, the company's core founding members had faculty and/or student roles at the university.

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